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VOL. 30

1952

FASC. 3—4

MERCATORIN KIRJAPAINO  
HELSINKI, FINLAND

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*Annales Medicinae Experimentalis et Biologiae Fenniae*

Yrjönkatu 17, Helsinki, Finland.

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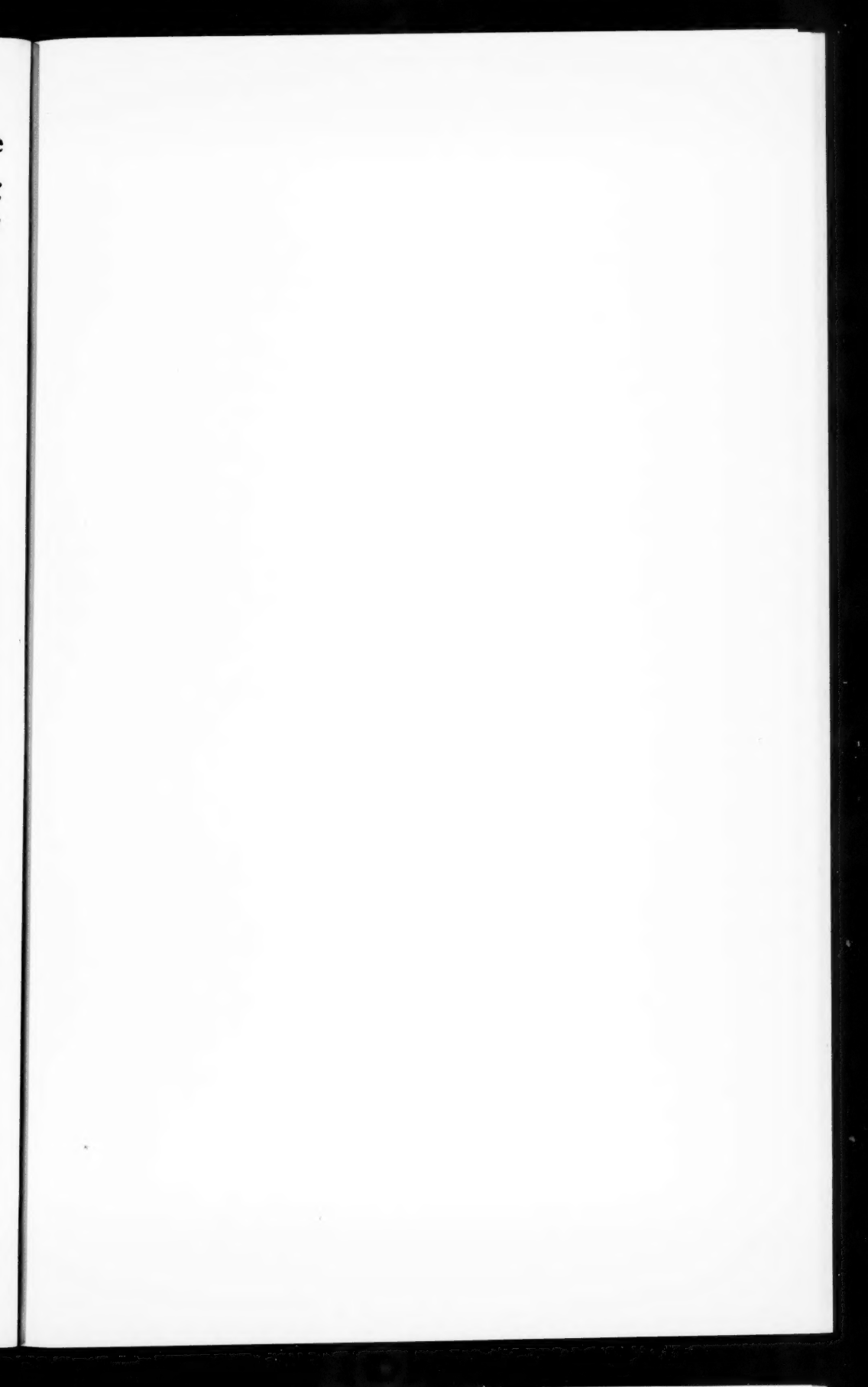
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## PAAVO EEVERTTI SIMOLA

M.D., PH. D.,

PROFESSOR OF MEDICAL CHEMISTRY

IN THE UNIVERSITY OF HELSINKI

It seldom happens that a homage volume is presented to a 50-year-old scientist. Such a thing is looked upon as something exceptional. But there is something exceptional about the career and personality of Professor Paavo Eevertti Simola, and it is above all as a sign of the esteem in which he and his achievements in medical science are held among his colleagues, pupils, and friends that this volume of studies is dedicated to him on his 50th birthday.

Son of a university professor, he submitted in the course of one year two doctoral dissertations, one to the Philosophical, one to the Medical Faculty, and was appointed to the chair of medical chemistry in the University of Helsinki at the age of 30. Since then, he has held several offices in the Medical Faculty. He was, among other things, Dean of the Faculty for nearly ten years.

His numerous publications give evidence of his scientific productivity. Above all, he has been interested in phenomena of intermediary metabolism, particularly in the metabolism of keto acids. He has also paid energetic attention to the study of the vitamins, hormones, and enzymes and to the methods of laboratory research. His familiarity with the different branches of medical chemistry — to keep up with the enormous progress made in this field is no easy task — has also greatly benefited the work carried out by others under his supervision.

Before Prof. Simola was appointed to the chair, medical chemistry was a neglected field in Finnish medical research. This is evident from the fact that the chair had been half a dozen years

without a holder. Prof. Simola's struggle for the recognition of the importance of medical chemistry for medicine is one of the notable features in his career as a university teacher. This and the fact that he has proved an excellent, inspiring teacher account for the large number of doctoral dissertations and other medical studies published under his supervision; it will hardly be an exaggeration to say that a special school of medical chemistry has developed around him.

Although Prof. Simola's main interests naturally lie in problems of a theoretical nature, he has by no means neglected to point out the importance of medical chemistry for practical medicine. Practical studies have been and are being carried out in his institute, and the present staffs of Finnish clinical laboratories have received their training under his guidance. His outstanding merits in the promotion of clinical laboratory research in the country are universally recognised. Yet he has also had time for activities outside personal research work and university teaching. He has been and is on the councils of several scientific societies and on several advisory committees. His election as Patron of the corporation of students from the district of Häme gives evidence of his popularity as a university teacher.

Those who are responsible for the present homage volume sincerely hope that Prof. Simola will accept it not only as a small token of the respect in which they hold him but also of their gratitude for what he has given them.

## THE PHOSPHORUS FRACTIONS IN HUMAN UTERINE MUSCLE

by

OLAVI KINNUNEN and AIMO PEKKARINEN

(Received for publication January 11, 1952)

### INTRODUCTION

The phosphorus content of smooth muscle has been relatively little studied, and chiefly in experimental animals. According to Walaas (13) rat and rabbit uterus, for example, contains very little of the high-energy phosphorus compounds, phosphocreatine (PC) and adenosinetriphosphoric acid (ATP) — no more than 1/50 and 1/20 respectively of the values determined for striated muscle. This accounts partly for the slowness of the contraction mechanism of smooth muscle (10, 4, 13). Estradiol and progesterone have no appreciable effect on these phosphorus fractions in smooth or striated muscle. However, estrogens increase the combination of radioactive P 32 with uterine acid-soluble P (14). Csapó (2) stated that the slowness and weakness of the uterine contraction mechanism is related also to actomyosin, of which uterine muscle contains less than striated muscle.

### THE OUTLINE OF THE PROBLEM

The metabolism of human smooth muscle is of considerable interest and we have therefore studied the phosphorus fractions

of the human uterus in cases of myoma and carcinoma and in cases of abdominal hysterotomy and caesarean section at the early and late stages of pregnancy.

#### MATERIAL AND METHODS

The phosphorus fractions have been determined from 52 human uteri. In 25 cases the uterus was removed because of myoma and in 4 cases because of carcinoma; in all these uterine samples the tissue was macroscopically normal.

The series further included 5 cases of abdominal hysterotomy for therapeutic abortion at the early stage of pregnancy and 18 cases of caesarean section at the end of pregnancy; 4 of these last were cases without pains.

The uterine muscle samples were obtained in connection with the operations.

Some difficulties were caused by the duration of operation and the operative procedure itself. The tissue specimens taken in connection with miniature and caesarean sections were rich in blood vessels. Seeing that the P fractions of blood differ greatly from those of uterine muscle (6, 7 a, b), the blood was at once carefully removed from these specimens. The tissue specimens were taken directly into liquid air or were kept at  $-3^{\circ}\text{C}$  and frozen with  $\text{CO}_2$ -ice. The mucosa was cut off. The frozen piece of muscle was dissected with a microtome. The methods were not founded to effect the phosphorus fractions.

For determination of inorganic phosphate P, ATP, hexose phosphate P, diphosphoglyceric acid P and total acid-soluble P one gram of finely ground tissue was extracted in a cooled mortar with 20 ml of ice-cold 5 per cent trichloroacetic acid. A piece of uterine tissue, 200 mg in weight, was digested with strong sulfuric acid and nitric acid in a Kjeldahl flask for determination of total P. The inorganic P and the PC were directly estimated according to Lowry and Lopez (9). The other phosphorus fractions and total phosphorus were determined by Fiske-Subbarow and Bomschow's method (1). The color intensity was estimated in a Stufen photometer with filter S 61. Another piece of 200 mg was dissected with a microtome and finely ground in a mortar containing Bloor's

solution for estimation of lipid P. After standing a few days the extract was digested with sulfuric acid and nitric acid, and the lipid phosphorus was determined by Kalaja's method (8).

#### RESULTS

*The Whole Material.* — The average P values  $\pm$  with 95 per cent confidence limit are presented in table 1. Phosphocreatine is an unstable compound and easily splits during operation. Thus it generally could not be demonstrated as a separate fraction, but only in the form of inorganic P. The second high-energy ester P, ATP, occurred in small and variable amounts in uterine tissue (5.4 mg per 100 ml), as did also hexose phosphate P (3.3 mg per 100 ml). Inorganic phosphate P (27.3 mg per 100 ml) and diphosphoglyceric acid phosphate P (20.9 mg per 100 ml) were present in fairly large amounts. Total acid soluble P (54.7 mg per 100 ml) and lipid P (45.3 mg per 100 ml) both represented about one-half of the total P content. On the whole the phosphorus fractions in this material varied considerably from case to case.

*The Individual Groups.* — The average  $\pm$  95 per cent confidence limits in the individual groups appear in table 2. The specimens of myomatous (I) or carcinomatous (II) but macroscopically normal non-pregnant uterus and the samples of pregnant uterus obtained at miniature (III) or caesarean section (IV) form the typical groups.

Caesarean sections in cases without pains (V) are also separately presented, but the number of these cases, as those of carcinomatous uterus, is too small.

The phosphorus values in specimens of myomatous and carcinomatous (non-pregnant) uterus were compared with the samples obtained at the end of pregnancy by caesarean section (table 3 VI); on the other hand the miniature section specimens were compared with the caesarean section specimens (table 3) (VII).

As regards inorganic phosphate the groups showed no distinct statistical differences. The results did not differ much from the average for the whole material. No statistical difference could be demonstrated in the content of ATP and hexose phosphate P. The average values for total acid soluble phosphorus and diphosphoglyceric acid P are approximately the same in all the groups.

TABLE 1  
PHOSPHORUS FRACTIONS OF HUMAN UTERUS (WHOLE MATERIAL)  
Number of Cases and Means (mg per cent)  $\pm$  95 per cent Confidence Limits

Inorganic Phosphate P	A T P	Hexose Phosphate P	Diphosphoglyceric Acid P	Total Acid-Soluble P	Total P	Lipid P
(51) 27.3 $\pm$ 1.9	(51) 5.4 $\pm$ 1.2	(47) 3.3 $\pm$ 1.2	(41) 20.9 $\pm$ 4.3	(46) 54.7 $\pm$ 5.6	(51) 108.7 $\pm$ 8.3	48) 45.3 $\pm$ 3.3

TABLE 2  
PHOSPHORUS FRACTIONS OF HUMAN UTERUS IN SPECIFIED GROUPS  
A = Non-pregnant uterus: I Myomatous II Carcinomatous  
B = Pregnant uterus: III Miniature section IV Caesarean section V Caesarean section without pains  
Number of Cases and Means (mg per cent)  $\pm$  95 per cent Confidence Limits

	Inorganic Phosphate P	A T P	Hexose Phosphate P	Diphosphoglyceric Acid P	Total Acid-Soluble P	Total P	Lipid P
A I	(25) 26.2 $\pm$ 2.2	(25) 5.5 $\pm$ 1.8	(23) 2.9 $\pm$ 1.9	(19) 21.3 $\pm$ 7.2	(24) 54.5 $\pm$ 7.9	(26) 111.8 $\pm$ 12.3	(25) 45.4 $\pm$ 5.1
II	(3) 23.5 $\pm$ 26.4	(3) 3.1 $\pm$ 7.0	(3) 1.1 $\pm$ 4.9	(4) 20.4 $\pm$ 19.7	(4) 48.6 $\pm$ 34.0	(4) 129.3 $\pm$ 28.6	(3) 47.1 $\pm$ 10.6
I+II	(28) 25.9 $\pm$ 2.3	(28) 5.2 $\pm$ 1.7	(26) 2.7 $\pm$ 1.7	(23) 21.2 $\pm$ 6.2	(28) 53.6 $\pm$ 7.6	(30) 114.1 $\pm$ 11.0	(28) 45.6 $\pm$ 4.5
B III	(5) 30.4 $\pm$ 6.2	(5) 5.6 $\pm$ 6.1	(4) 6.7 $\pm$ 6.6	(2) 22.5 $\pm$ 24.2	(3) 58.2 $\pm$ 17.4	(4) 130.7 $\pm$ 35.9	(2) 52.2 $\pm$ 172.5
IV	(14) 29.1 $\pm$ 5.1	(14) 4.9 $\pm$ 2.7	(14) 3.5 $\pm$ 2.3	(12) 21.1 $\pm$ 8.4	(12) 56.1 $\pm$ 13.0	(13) 96.7 $\pm$ 17.2	(14) 40.8 $\pm$ 4.6
V	(4) 27.6 $\pm$ 12.9	(4) 8.6 $\pm$ 5.3	(4) 2.8 $\pm$ 3.4	(3) 16.5 $\pm$ 38.4	(3) 55.6 $\pm$ 57.9	(4) 85.7 $\pm$ 19.9	(4) 55 $\pm$ 21.4
IV+V	(18) 28.7 $\pm$ 4.2	(18) 5.7 $\pm$ 2.3	(18) 3.4 $\pm$ 1.8	(15) 21.3 $\pm$ 7.0	(15) 56.0 $\pm$ 11.1	(17) 94.1 $\pm$ 13.2	(18) 44 $\pm$ 5.4

TABLE 3  
Comparison of Phosphorus Fractions in Specified Groups ( $\Delta$  = Difference of Means, tp = Probability)

	Inorganic Phosphate P	A T P	Hexose Phosphate P	Diphosphoglyceric Acid P	Total Acid-Soluble P	Total P	Lipid P
	$\Delta$ tp	$\Delta$ tp	$\Delta$ tp	$\Delta$ tp	$\Delta$ tp	$\Delta$ tp	$\Delta$ tp
VI	2.8 1.33	0.5 0.38	0.6 0.52	0.2 0.04	2.4 0.38	20.0 2.8 <sup>1</sup>	1.6 0.48
VII	1.6 0.41	0.1 0.06	3.3 1.62	1.2 0.12	2.2 0.18	6.6 2.60 <sup>1</sup>	8.2 0.96

1 0.05 - 0.01 per cent significant.

They showed no statistically significant differences in the individual groups.

However, a comparison of the average values for total phosphorus showed a significant difference: the total P content was smaller at the end of pregnancy than in the non-pregnant uterus or in the early stages of pregnancy.

With regard to lipid phosphorus the differences between the groups were not statistically significant.

#### CONCLUSIONS

The phosphorus fractions of man as studied on operatively removed uterine specimens are perhaps more inconstant than those demonstrated by animal studies: the conditions under which the specimens were taken are not entirely comparable owing to the duration of operation. Thus the phosphorus fractions of the human uterus cannot give an equally good picture of the P metabolism as the values determined for animals.

The phosphorus fractions in the human uterus were much the same as the values reported for rabbit and guinea pig uterus (13).

The principal P fractions in human uterine muscle were, on the basis of this study, inorganic P, diphosphoglyceric acid P and lipid P. Thus the human uterine muscle contains little of the high-energy phosphorus compounds, i.e. PC and ATP, of which striated muscle contains large amounts. Phosphocreatine could not generally be demonstrated on this material, but this fraction was also very small in the uterine tissue of experimental animals (3, 11, 13). Our values for ATP and hexose phosphate P were also very low.

No statistically significant differences were noted in the phosphorus fractions, with the exception of total P, which was found to be low at the end of pregnancy as compared with the non-pregnant uterus and with specimens from the early stages of pregnancy. It is possible that more phosphorus is consumed, but changes in the fluid and mineral content of tissues may also contribute. In the later stages of pregnancy many tissues, also the uterus, show a tendency to edema (11, 12).

## SUMMARY

The phosphorus fractions have been determined from 52 human uteri. The series included 25 cases in which the uterus was removed because of myoma and 4 in which it was removed because of carcinoma; in all these samples the tissue was macroscopically normal.

In addition uterine tissue samples were obtained in 5 cases of abdominal hysterotomy in the early stages of pregnancy and 18 cases of caesarean section at the end of pregnancy; 4 of these last were cases without pains.

In the whole series the average P-values were as follows: inorganic phosphate 27.3 mg per 100 ml, adenosinetriphosphoric acid 5.4 mg per 100 ml, hexose phosphate 3.3 mg per 100 ml, diphosphoglyceric acid 20.9 mg per 100 ml, acid soluble phosphorus 54.7 mg per 100 ml, lipid phosphorus 45.3 mg per 100 ml, and total phosphorus 108.7 mg per 100 ml. In some cases there was no adenosinetriphosphoric acid and/or no hexose phosphate.

A comparison of the phosphorus fractions in all these groups revealed a statistically significant difference in the content of total phosphorus alone; it was somewhat lower at the end of pregnancy than early in pregnancy or in the non-pregnant state.

## REFERENCES

1. BOMSKOW, C.: *Ztschr. f. physiol. Chemie*, 1932:210:67.
2. CSAPÓ, A.: *Acta Physiol. Scand.* 1949:19:100.
3. EGGLETON, P., and EGGLETON, G. P.: *J. Physiol.* 1929—30:68:193.
4. FISCHER, E.: *Physiol. Rev.* 1944:24:467.
5. FISKE, C. H., and SUBBAROW, J.: *J. Biol. Chem.* 1925:66:375.
6. HELVE, O.: *Acta Med. Scand.* 1946:125:543.
7. HELVE, O.: *Acta Soc. Med. Fenn. Duodecim Ser. A. Tom. XXIV. Fasc. 3*, 1946:124.
8. KALAJA, T., and VESA, A.: *Acta Soc. Med. Fenn. Duodecim Ser. A. XXI, Fasc. 1*, 1939.
9. LOWRY, O. H., and LOPEZ, J. A.: *J. Biol. Chem.* 1946:162:421.
10. LUNDGAARD, E.: *Biochem. Ztschr.* 1930:227:51.
11. SBUTEGA, U., and VESZELKA, F. DE: *Rassegna d'ostet.ginec.* 1936:45:407.
12. SEITZ, L., and AMREICH, A. I.: *Biologie u. Pathologie des Weibes.* Urban & Schwarzenberg, Berlin u. Wien, 1944.
13. WALAAS O., and WALAAS, E.: *Acta Physiol. Scand.* 1950:21:1.
14. WALAAS, O., and WALAAS, E.: *Ibid.* 1950:21:18.

## ÜBER DAS MESSEN DER WAHRZUNEHMENDEN »REINEN« GESCHWINDIGKEIT

von

YRJÖ REENPÄÄ

(Bei der Schriftleitung eingegangen am 10. März 1952)

Die Lehre von der Extension und Intension der Zeichen, welche Carnap (1) entwickelt hat, und die in gewissem Sinne auf Frege zurückgeht, kann als eine Weiterentwicklung der Lehre Kant's (4) von der Dualität des menschlichen Verstandes aufgefasst werden. Nach Kant besteht derjenige Teil unseres Verstandes, welcher von ihm der reine Verstand genannt wird, aus der reinen Anschauung (Zeit und Raum), und aus reinem Denken oder reiner Begrifflichkeit (die Kategorien), welche beiden Stämme unseres Intellektes aus einer gemeinsamen Wurzel, »der reinen Einbildungskraft« entspringen. Die Lehre von der Extension und Intension der Zeichen hat in ihrer Art den Gedanken der Doppeltheit der Stämme des Verstandes aufgenommen, obwohl diese Ähnlichkeit bei den Darlegungen der modernen Extensions-Intensions-Lehre nicht erwähnt worden ist. Diese neue Lehre besitzt auch nicht denjenigen Bestandteil einer Verstandeslehre, welche in dem Kant'schen System als Wurzel unseres reinen Verstandes bezeichnet wird und welche wohl Begründung, Basis oder Grund des Denkens über das Denken und Anschauen sein muss. Dieser Mangel einer Begründung der Dualität der Objekte der Zeichen in der Extensions-Intensions-Lehre dürfte auch teilweise damit im Zusammenhang stehen, dass in ihr die Frage der Objekte unentwickelt ist. In Kants Lehre ist die Objektenfrage auf der Seite der reinen Anschauung klar entschieden mit der Aufstellung der »Objekte« des Raumes und der Zeit. In

anderer Terminologie könnten wir diese Objekte »reine« Phänomene nennen. Auf der Seite des reinen Denkens hat Kant die Objektenfrage mit Hilfe seiner Kategorien zu beantworten versucht in einer, unseres Erachtens, ganz fundamentalen Weise. Kant's Lehre vom reinen Verstand dürfte als Grund einer Lehre vom »Verstand überhaupt«, einer Lehre des empirischen Verstandes, eben in betreff der Objektenfrage weiter ausgebaut werden können, mit Hilfe der Technik der Extensions-Intensions-Lehre und mit dem Zusatz der empirisch phänomenalen Objekte, der »Sinnlichkeit a posteriori«, zu den »reinen Phänomena« des Raumes und der Zeit.

Heidegger (2) hat in seiner Interpretation von Kant, gezeigt, wie die reine universale Anschauung Kants, seine »Zeit«, ein Phänomenon ist, von der Art des »Vorhandenen«. Die vorhandene Phänomenalität Heidegger's umfasst die uns nicht vital angehende Welt, die sich uns als Objekt des Wahrnehmens ergibt. Diese Mannigfaltigkeit des Vorhandenen umfasst manche derjenigen »Dinge«, die vorhanden, aber nicht zuhanden sind, also zum Beschauen da, aber nicht zuhanden sind. Die Ganzheit der vorhandenen Welt dürfte von dem von uns beschriebenen Zusammenhang der verschiedenen Sinnesmodalbereiche gebildet sein, mit ihren gemeinsamen, intermodalen Zeit- und Lokaldimensionen [Reenpää (7)]. Die positivistische Empfindungswelt Mach's (6) hat in dieser Weise, als *Mannigfaltigkeit des Vorhandenen*, ihren Platz in dem All des uns sich Ergebenden. Im Gegensatz zur vorhandenen Welt, der Welt der Wissenschaft, beschreibt Heidegger des weiteren den Bereich des »Zuhandenen«. Zu ihm gehören die Objekte vom Charakter des Zeuges, die sich uns ihres Gebrauches wegen dargeben. So ist der Hammer zum Hämmern zuhanden, die Nadel zum Nähen, obwohl sie beide auch vorhandene Dinge der wissenschaftlichen Expectation sein können. Auf der Basis von Kant's Lehre von der Dualität des reinen Verstandes, erweitert mit den Objekten der empirischen Sinnlichkeit, behandelt mit der sauberen Technik der symbolischen Logik und unter dem Aspekten der tiefen Erschlossenheit der Heidegger'schen Kant-Interpretation, dürfte man versuchen können, aufs neue das Basisproblem der Beobachtungswissenschaften aufzunehmen.

Hier beschränken wir uns aber auf die Behandlung zweier Kant-Heidegger'schen Vorhandenheiten, auf diejenigen der erlebten *Zeit* und der erlebten *Strecke*. Dem Zeiterlebnis »entspricht« der

Zeitbegriff, wobei dem »Entsprechen« die Kant-Heidegger'sche Formulierung gegeben werden kann, dass die reine Anschauung, das Zeiterleben und andererseits das reine Denken, der Zeitbegriff, als zwei Stämme des Zeitverständnisses, aus der einen gemeinsamen Wurzel, der »ursprünglichen Zeit« entsprungen sind. Heidegger's ursprüngliche Zeit entspricht hier der reinen Einbildungskraft Kant's und kann uns vielleicht zum Verständnis darüber verhelfen, wie die ursprüngliche, für das Leben *zuhandene* Zeit sich in die beiden genannten Stämme verzweigt hat.

Die Extensions-Intensions-Lehre von Carnap gibt unserem »Entsprechen« eine andere Formulierung. Einem Zeichen  $t$  entsprechen zwei Designata, zwei Objekte, einerseits das Zeiterlebnis, welche die Extension des Zeichens ist, und andererseits der Zeitbegriff, der seine Intension ist. Die nähere Ausführung und Begründung der Extensions-Intensions-Dualität bei den Basis-Objekten hat Reenpää (8) früher gegeben und hierbei die Zeitlichkeit, die Aktualität als das die Extensionen charakterisierende, das Zeithobene als das die Intensionen charakterisierende aufgestellt. So ist z.B. die Extension des Zeichens »b« (blau) das aktuelle, zeitliche Blauerlebnis, wogegen seine Intension der zeithobene Begriff des Blauen ist. Bei allen Objekten, die zu den Vorhandenheiten der Basis-Stufe der Erlebnismannigfaltigkeiten gehören, kann man durch Abziehen der Zeitlichkeit von der aktuellen Extension die zeitlose Intension erhalten. Die Intension des Zeichens  $b$ , z.B., ist ein Nur-Blau, ein Denkprodukt, Begriff, das aus der empirischen »Sinnlichkeit« des Blauerlebens »deduziert« worden ist. Da die Zeitlichkeit das Scheidende zwischen der Extension und Intension ist, wird die Intension des Zeichens der Zeit  $t$  eine ausgezeichnete Stellung unter den Übrigen Intensionen der Basis-Objekte erhalten. Wenn von der Extension des Zeit-Zeichens, dem Zeiterlebnis das aktuelle Zeitliche abgezogen wird, also von dem Zeiterlebnis das Zeiterlebnis selbst weggenommen wird, ist das Übrigbleibende eigentlich eine »Leerheit«, welche der Zeitbegriff genannt wird. Diese »zeithobene Zeit« ist die Intension des Zeit-Zeichens. Aber nicht nur in dieser, sondern auch in anderen Hinsichten, wird sich die Eigenartigkeit der Zeitlichkeit unter den übrigen Basis-Objekten zeigen. Die Zeit als »reine *universale* Anschauung« wird ihre Universalität auch bei einer Analyse dieser Art hervortreten lassen.

Das andere Objekt der »reinen Anschauung«, der Raum, hat nicht den universalen Charakter der Zeitlichkeit. Dies kommt auch darin zum Ausdruck, dass die Modalmannigfaltigkeiten unserer verschiedenen Sinne kaum alle eine gemeinsame Raum- oder besser Lokaldimension haben, während sie alle eine gemeinsame »Zeitachse« besitzen. Anders ausgedrückt: ein jedes Erlebnis vom Vorhandenheitscharakter hat eine Zeitkomponente; die Extensionen der Zeichen der »empirischen« Sinne (der »Sinnlichkeit a posteriori«) sind Zeitaktualitäten, wogegen die Erlebnisobjekte eine unbestimmte Lokalität haben können. Man könnte die Sache so ausdrücken, dass die Lokalität gewissermassen eine Zwischenstellung innehat, zwischen einerseits der universalen Zeitlichkeit und andererseits den speziellen Sinnesqualitäten; alles Erlebte ist zeitlich, aber oft nur in unklarer Weise räumlich oder lokalisiert. In Locke'scher (5) Terminologie könnte man sagen, dass die Zeit eine unbedingt primäre Qualität ist, der Raum aber nur eine bedingt primäre. Wegen dieser Eigenheit des Raumes ist es auch nicht angezeigt, die Räumlichkeit oder die Lokalität zum Scheidenden der Extension und Intension der Zeichen der Basis-Objekte zu nehmen, sondern die universale Zeitlichkeit hier walten zu lassen. Demgemäss ist die Extension des Zeichens der Lokalität, z.B. einer Strecke, *s*, das Streckenerlebnis, welches ein solches des Gesichtes, des Getastes oder des Bewegens sein kann, und die Intension dieses Zeichens der Streckenbegriff, wie er in der Geometrie und der Physik vorkommt. Das Unterscheidende der Extension von der Intension ist hier, ganz wie bei den sekundären, a posteriori Sinnlichkeiten, die aktuelle Sinnlichkeit; das Streckenerlebnis ist eine Aktualität, der Streckenbegriff eine zeitenthobene Intension.

Auf dem Grund dieser prinzipiellen Darlegungen wollen wir nun untersuchen, wie ein paar Ergebnisse der Zeit- und Strecken- oder Längenmessungen, insbesondere diejenigen, die Geschwindigkeitsmessungen genannt werden können, von unserem Standpunkt aus aufzufassen wären. Zu diesem Zweck bezeichnen wir eine Strecke mit dem Zeichen *s*, wobei die Extension dieses Zeichens ein Streckenerlebnis ist, dessen Teilschaft zum Gesicht, Getast oder zu einem anderen Sinne bis auf weiteres unbestimmt bleibe; die Intension des Zeichens ist der Streckenbegriff der Physik und Geometrie. Um es deutlich zu machen, wann wir die Extension und wann die Intension von *s* meinen, bezeichnen wir die Extension mit *e<sub>s</sub>* und

die Intension mit  $i_s$ . Wie alle phänomenalen Dimensionen der Modalmannigfaltigkeiten, kann auch bei den Dimensionen der Strecken- und der Zeiterlebnisse, wegen ihrer eigenartigen Diskontinuität, von Minimal- oder Schwellenwerten gesprochen werden; diese bezeichnen wir mit  $e_{s_u}$  bzw. mit  $e_{t_u}$ . Die diesen Extensionen entsprechenden Intensionen, die begrifflichen, sog. Reizschwellen, bezeichnen wir mit  $i_{s_u}$  bzw. mit  $i_{t_u}$ .

Wir wollen erstens die *elementaren* oder *schwellenmässigen* oben genannten Extensions- und Intensionsgrössen miteinander vergleichen. Wenn eine Strecke in der Weise eines »Durcherlebens« erlebt wird, wenn z.B. im Gebiet des Tastsinns sich etwas von einer Stelle auf der Haut zu einer anderen Stelle bewegt und dieses in der Zeit erlebt wird, oder wenn beim Bewegen eines Gliedes die Bewegungsstrecke durcherlebt wird, ist diese Erlebnis-Extensionalität immer mit einem Zeiterlebnis verbunden, d.h. die Erlebnisse der Zeit und der Strecke geschehen »miteinander«. Dies gilt natürlich auch in betreff der Schwellen-Extensionalitäten  $e_{t_u}$  und  $e_{s_u}$ . Das Durcherleben der Schwellen-Extensionalität in der Schwellenzeit kann als ein Geschwindigkeitserlebnis bezeichnet werden und in gewissem Sinne als ein *Grenz-* oder *Singulär*geschwindigkeitserlebnis, weil eine kleinste zu erlebende Strecke in einer kleinsten zu erlebenden Zeit durcherlebt wird. Wir können diese Extensionalität des Geschwindigkeitserlebnisses mit  $e_{s_u} : e_{t_u}$  bezeichnen. Man muss beachten, dass dieses Geschwindigkeitserlebnis nicht in dem Sinne ein Gleichzeitigkeitserlebnis von einer Länge und einer Zeit ist, dass ein Streckenerlebnis während einer gewissen Zeit, z.B. einer Zeitschwelle, vorhanden wäre, wie dies z.B. beim Betrachten einer in ihrer Ganzheit sichtbaren Strecke auf dem Gebiete des Gesichts der Fall ist. Unsere obige Zeichenreihe designiert die extensionale Grösse des Geschwindigkeitserlebnisses, des Durcherlebens oder Durchlauferlebens der Strecke in der Zeit.

Die intensionale Grösse  $i_{s_u}$  bezeichnet die der kleinsten zu erlebenden Strecke entsprechende geometrische Streckengrösse, deren Grösse mit Hilfe einer arbiträren Masszahl (gemessen z.B. in Zentimetern) angegeben ist. Entsprechendes gilt in betreff der intensionalen Grösse  $i_{t_u}$ , deren Grösse z.B. in Sekunden angegeben sein kann. Der Extensionalität des Geschwindigkeitserlebnisses, wie wir es dargestellt haben, »entspricht« nun die Intensionalität des begrifflichen Verhältnisses der Masszahlen von  $i_{s_u}$  und  $i_{t_u}$ , also das Ver-

hältnis  $i s_u : i t_u$ , welches eine begriffliche Geschwindigkeit ist.<sup>1</sup> Da  $i s_u$  und  $i t_u$  beide relativ konstante Werte aufzeigen, wenn ihre Messung in einem bestimmten Modalbereich und in genau bestimmter Art des »Durcherlebens« ausgeführt wird, ist auch ihr Verhältnis eine Konstante. Die Behauptung, dass die genannten Werte relativ konstant sind, setzt eine Wiederholung der Bestimmungen voraus, also etwas, dessen Behandlung hier nicht ausgeführt ist; die Kürze dieser Abhandlung erlaubt nicht hierauf einzugehen. Wir bezeichnen diese Konstante mit  $k$ , wobei ihre Grösse natürlich von dem Modalbereich und von der Art des »Durcherlebens« (sowie, was selbstverständlich ist, auch von der Wahl der arbiträren Einheiten der Strecken- und Zeitmasse) abhängt. Dem »Durchlaufserleben« und dessen Zeichen  $e s_u : e t_u$  entspricht also die begriffliche Durchlaufgeschwindigkeit und dessen Zeichen  $i s_u : i t_u = k$ , in welcher Gleichung  $k$  also verschiedene Werte haben kann. (Wie im Anfang dieser Abhandlung dargelegt wurde, kann das Durcherleben auch der kleinsten Länge nicht momentan erfolgen; die aktuelle Zeitlichkeit ist der »Rechtsgrund«, das sine qua non des anschauungsmässig Erlebhaften. Die extensionale Zeit  $e t_u$  kann also nicht ohne Dauer sein und die intensionale Zeit  $i t_u$  kann nicht den Zahlenwert Null haben. Hiernach muss der Wert der Geschwindigkeit kleiner als unendlich sein;  $i s_u : i t_u < \infty$ ).

Wir wenden uns nun zur Betrachtung der *überschwelligen* Strecken und Zeiten und zeigen, wie eine zweite intensionale Gleichung dieser Grössen hergeleitet werden kann. Auf Grund des Vorhindargelegten verstehen wir, dass die Zeichenreihe  $e s : e s_u$  das erlebnismässige Durchmessen der Strecke  $e s$  mittels der Minimalstrecke  $e s_u$  bedeutet, sie gibt das Nacheinandererleben der im Überschwellererleben einhergehenden Schwellenerlebnisse wieder. Wir haben dieses extensionale Messen früher das Eigenmessen genannt. Das Entsprechende Eigenmessen in betreff der Zeit wird durch die Zeichenreihe  $e t : e t_u$  wiedergegeben. Wenn nun sowohl die Strecke  $e s$  als die Zeit  $e t$  »gleichzeitig durchgegangen« werden, muss bei diesem *gemeinsamen* Durcherleben der beiden überschwelligen Phänomenalitäten einem jeden Streckenminimalerlebnis wenigstens ein Zeitminimalerlebnis entsprechen, denn, wenn einem Zeit-

<sup>1</sup> Die Demonstration der »Entsprechung« kann in der von uns [Reenpää (9)] dargegebenen Weise vorgenommen werden. Sie soll in anderem Zusammenhang dargelegt werden.

minimalerlebnis eine längere Strecke als diejenige des Streckenminimalerlebnisses entspräche, würden die Teile dieser Strecke in Zeiten, die kürzer als diejenige des Zeitminimalerlebnisses sind, »erlebt« werden müssen, was nicht möglich ist, da das Zeitminimalerlebnis die kürzeste zu erlebende Zeit ist. Da die Teile der Strecke in diesem Falle also nicht erlebnismässig, d.h. anschauungsmässig sind, ist es auch die ganze Strecke nicht. Anschauungsmässig, extensional sein, ist eben dasselbe wie in der Zeit erlebt werden. Die längste »gemeinsam« mit einem Zeitminimalerlebnis oder »während« eines Zeitminimalerlebnisses zu *erlebende* d.i. *anschauungsmässige*, extensionale Strecke, ist also die des Streckenminimalerlebnisses. Hieraus folgt, dass beim Durchleben oder Durchschreiten einer Strecke, die Anzahl der Längenschritte kleiner oder höchstens gleich sein kann der Anzahl der durchwanderten Zeit-Schwellen; bezeichnet  $e^s : e^{s_u} \leq e^t : e^{t_u}$ . Das Gleichheitszeichen gilt hier für den Fall des am schnellsten vorsichgehenden Durchlaufens, das Ungleichheitszeichen für ein langsames Durchlaufen, also für ein Durchlaufen, bei dem einem oder mehreren Längenschritten mehr als eine Zeitschwelle entspricht. Wir können diese Zeichenreihe als eine Extensionalität ansehen, wenn das Eigenmessen zu diesem Stamm des Verstandes gerechnet wird.

Die entsprechende intensionale Zeichenreihe ist  $\frac{i^s}{i^{s_u}} : \frac{i^t}{i^{t_u}} \leq 1$ .

Hier entspricht der Extension des Durchlaufens (der Eigenmessung, :) die Intension des Dividierens (:) und den extensionalen Strecken- und Zeitgrössen entsprechen ihre früher dargelegten intensionalen Begrifflichkeiten, wobei diese mit gewissen, arbiträren Masseinheiten gemessen sind. Die Zeichenreihe besagt: Wenn eine Strecke ( $i^s$ ) mit der, der kleinsten zu erfahrenden Streckengrösse ( $e^{s_u}$ ) entsprechenden begrifflich bestimmten Strecke ( $i^{s_u}$ ) gemessen wird und »gleichzeitig« die dazu erforderliche Zeit ( $i^t$ ) mit dem, der kleinsten wahrzunehmenden Zeit ( $e^{t_u}$ ) entsprechenden Zeitmassstabe ( $i^{t_u}$ ), so ist die Masszahl der Strecke kleiner oder höchstens gleichgross (von der Art und Modalität des Messens abhängig) wie die Masszahl der Zeit. Die obige intensionale Begriffsgleichung gibt der extensionalen Anschauungswirklichkeit Ausdruck, dass beim extensionalen, wirklichen Messen (beim Eigenmessen), keine Streckenelemente ohne entsprechende Zeitelemente auftreten können.

Wir haben nun zwei auf Anschauungswirklichkeiten sich basierende, intensionale Ausdrücke aufgestellt, und wollen sie nochmals nebeneinander aufschreiben:

$$(1) \quad {}_i s_u : {}_i t_u = k$$

$$(2) \quad \frac{{}_i s}{{}_i s_u} : \frac{{}_i t}{{}_i t_u} \leq 1$$

Wenn man die Ausdrucksweise von Kant heranziehen würde, könnte man sagen, dass die Begrifflichkeiten dieser Zeichenreihen Verstandesbegriffe sind, die aus der reinen Anschauung des Raumes und der Zeit *deduziert* worden sind. Auch könnte man die Sachlage so ausdrücken, dass die von den Zeichenreihen dargegebenen Verstandesbegriffe in der Zeit-Raum-Anschauung ihre *Schemata* haben. Beide Ausdrucksweisen, sowohl diejenige des Deduzierens der Verstandesbegriffe aus der Anschauung, als diejenige des Gründens des Verstandes in Anschauungs-Schemata oder Anschauungs-Bildern, erläutern in verschiedener Weise den Kantischen Gedanken von den beiden Stämmen unseres Verstandes. Wenn die Extensions-Intensions-Lehre in der von mir (8) gegebenen Weise verstanden wird, was mit Hilfe ihrer Applikation auf die Grund-Objekte der Vorhandenheiten geschehen kann, fällt die Dualität der Extension-Intension der Zeichen mit den beiden Stämmen des Anschauendenkens, des Verstandes zusammen. Und demgemäss kann das hier behandelte Problem der reinen Anschauung und des daraus »deduzierten« Denkens, welches zum Kantischen Problemkreis gehört, auch mit Hilfe der Extensions-Intensions-Lehre dargestellt werden.

Wenn die linken Seiten und entsprechend die rechten Seiten der obigen zwei Ausdrücke miteinander multipliziert werden [die Seiten des Ausdrucks (2) sind Zahlen], erhalten wir den Ausdruck:

$$(3) \quad {}_i s : {}_i t \leq k$$

Hier bedeuten also  ${}_i s$  und  ${}_i t$  Längen- bzw. Zeitbegrifflichkeiten, die je mit einem arbiträren Masstab gemessen sind. In diesem Ausdruck fehlen die Zeichen der Schwellengrössen und er besagt demgemäss etwas über das »gewöhnliche«, nur mit Hilfe von arbiträren Masseinheiten geschehende Strecken- und Zeitmessen. Wenn wir berücksichtigen, dass der Intension des Dividierens (bezeichnet :) die Extension des gemeinsamen Durchschreitens oder Durcherlebens (bezeichnet :) entspricht, so können wir dem obigen Ausdruck den

folgenden Wortlauf geben: *Das Verhältnis einer Längengrösse zur gemeinsam mit ihr gemessenen Zeitgrösse ist immer unterhalb oder höchstens gleich einer endlichen Konstante.* Das was wir hier ein gemeinsames Messen genannt haben, das durchlaufende oder durcherlebte Messen einer Strecke in der Zeit, dürfte aber auch eine *physikalische* Geschwindigkeitsmessung genannt werden können. Kann nun aber dem obigen Ausdruck die Formulierung gegeben werden, dass eine jede physikalische Geschwindigkeitsmessung einen Zahlenwert ergeben muss, welcher höchstens einen bestimmten Wert ( $= k$ ) erhalten kann? Diese Frage muss deswegen genau untersucht werden, weil die Deduzierung der obigen Gleichung aus reinen Anschauungsdaten erfolgte, aus Daten, die wir gewöhnlich, wenigstens in dieser Weise, nicht zur empirischen oder wahrnehmungsmässigen Basis der Physik mitrechnen.

Die beiden Zeichenreihen (1) und (2) sind zeithobene, intentionale »Deduktionen« von zeitlichen, extensionalen Erlebniszusammenhängen. Bei der Reihe (1) ist dieser Erlebniszusammenhang ein schwellenmässiger und bei der Reihe (2) ist er ein solcher des Durcherlebens von überschwelligen Erlebnissen. In beiden Fällen beschrieben wir das Erleben als ein monomodales, als auf nur einem Sinnesgebiete stattfindendes. Besonders wurde das Geschehen des Durcherlebens der Zeichenreihe (2) als auf dem Gebiete des Getastes oder des Sich-Bewegens dargestellt. Wesentlich für die Reihe (2) ist aber die Monomodalität nicht, denn ersichtlich muss eine Zeichenreihe von ähnlicher Form bei einem jeden Strecken-Durcherleben gelten, unabhängig davon, in welcher Art das Durcherleben vorsichgeht, wenn es nur ein Erleben ist, denn die Universalität der Schwellen der Zeitanschauung, d.h. dasjenige Verhalten, dass einer jeden Streckenschwelle wenigstens eine Zeitschwelle entsprechen muss, gilt ganz allgemein. Auch die Zeichenreihe (1) muss im Falle einer nicht monomodalen Durcherlebens-Art, auf der rechten Seite der Gleichung einen bestimmten konstanten Wert von  $k$  haben, denn das »Durchlaufen« einer irgendwie durchzugehenden Strecke muss eine kleinste Durchlaufszeit besitzen.

Die aus den Zeichenreihen (1) und (2) hergeleitete Reihe (3) ist also eine Intensionalität, die für eine jede wirklich, d.h. im Durcherleben einer Länge in der Zeit, durchgeführte Geschwindigkeitsmessung gilt. Ein solches Geschwindigkeitsmessen dürften wir aber

als eine »reine« Messung, weil auf die Anschauungsformen des Raumes und der Zeit bezogene, aber auch als ein physikalisches Geschwindigkeitsmessen benennen können, im Gegensatz zum intensionalen, zeitenthobenen, nicht »durcherlebten« Geschwindigkeitsmessen, z.B. der Kinematik. Das erstgenannte, empirische Messen gaben wir durch das Zeichen  $\vdots$  wieder; das Zeichen des intensionalen Dividierens ist : .

Die Aussage der Zeichenreihe (3), dass bei einer extensionalen, »reinen« Geschwindigkeitsmessung der Wert der Geschwindigkeit einen Höchstwert haben muss, ist also als eine Folge der innewohnenden Eigenheiten unserer Zeit- und Längenerlebnisse, der Formen unserer reinen Anschauung, dargetan worden. Die von Kant hervorgehobene Universalität der reinen Anschauung der Zeit im Verhältnis zu allen anderen Formen der Anschauung a priori und a posteriori, auch derjenigen des Raumes (der Strecken), ist der letzte Grund dieses Verhaltens.

Schliesslich soll hervorgehoben werden, dass das dargelegte »reine« Prinzip der Höchstgeschwindigkeit nichts über den Wert dieser Geschwindigkeit aussagt. Dass dieser in der Physik erfahrungsgemäss gleich der Lichtgeschwindigkeit ist, gehört nicht zum »rein« Anschauungsmässigen im a priori'schen Sinn, sondern ist ein empirisches Ergebnis, man möchte sagen eine Errungenschaft a posteriori. Es ist vielleicht doch eigenartig, dass unser Ausdruck (3), der die gegenseitige Abhängigkeit der »anschauungsmässigen« Höchstgeschwindigkeit und der Grössen der *Elementarschritte* der Länge und der Zeit angibt (gemäss Gleichung (1) ist die Höchstgeschwindigkeit  $k = i s_u : i t_u$ ), eine gewisse Entsprechung in der physikalischen Theorienbildung hat. Wenn der »Elektronenradius« ( $\Lambda$ ) mit der physikalischen Höchstgeschwindigkeit, der Lichtgeschwindigkeit ( $c$ ) dividiert wird, ist das Ergebnis eine Zeitgrösse, als »Elementarzeit« ( $\tau$ ) bezeichnet ( $\Lambda : c = \tau$ , oder, der Analogie halber  $c = \Lambda : \tau$ ).

## LITERATUR

1. CARNAP, RUDOLF: *Meaning and Necessity*. The University of Chicago Press, Chicago, Ill, 1947.
  2. HEIDEGGER, MARTIN: *Kant und das Problem der Metaphysik*. Friedrich Cohen, Bonn, 1929.
  3. JORDAN, PASCAL: *Die physikalischen Weltkonstanten*. Naturwissenschaften, Jhrg. 25, S. 513, 1937.
  4. KANT, IMMANUEL: *Kritik der reinen Vernunft*. Reclams Universal-Bibliothek N:o 6461—70, Leipzig, 1944.
  5. LOCKE, JOHN: *An Essay Concerning Human Understanding*. Abridged and Edited by Raymond Wilburn. J. M. Dent & Sons, Ltd. London, 1947.
  6. MACH, ERNST: *Analyse der Empfindungen*. 3. Auflage, G. Fischer, Jena, 1902.
  7. REENPÄÄ, YRJÖ: *Über Wahrnehmen, Denken und messendes Versuchen*. E. J. Brill, Leiden, 1947.
  8. REENPÄÄ, YRJÖ: *Die Dualität des Verstandes*. Sitzungsber. d. Heidelberger Akad. d. Wiss. Math.-Naturwiss. Klasse. 7. Abhandlung, Springer, Heidelberg, 1950.
  9. REENPÄÄ, YRJÖ: *Der Verstand als Anschauung und Begriff*. Annal. Acad. scient. Fennicae. Ser. B. T. 76,1, Helsinki, 1952.
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## INFLUENCE OF THE NITROGEN CONTENT OF CELLS ON THEIR ENZYMATIC ACTIVITY<sup>1</sup>

by

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(Received for publication March 20, 1952)

The starting point for the investigations on the dependence of the enzyme activity of cells on their nitrogen content was the idea advanced by me in 1942 in a short paper »Die Enzyme in lebenden Zellen» in the journal of the Finnish Chemical Society (1). This idea was that the total protein of young active cells is practically enzyme protein and that the nitrogen content of cells is a limiting factor in enzyme formation.

In the same paper the hypothesis was advanced by me that the enzymes, which are needed in the first reaction with the corresponding substrates, are located on the surface of the cells. A substrate can be used as a nutrient only if the corresponding enzyme is to be found on the surface of the cell. The »affinity» between the enzyme and the substrate is the power which attracts the substrate to the cell. The other enzymes which are needed in the later metabolism of the substrate are located so that the reaction can continue from the first enzyme on. The location of the enzymes in the cell should thus regulate the metabolism. According to this hypothesis the uptake of nutrition should be a series of enzymatic reactions, which begin right on the surface of the cell.

If the concept that the protein content of cells is a limiting factor in enzyme formation holds good, it is to be expected that as the nitrogen content of cells falls either all the enzymes lose the corresponding part of their activity (calculated per dry matter),

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<sup>1</sup> Presented in the symposium held in connection with the Diamond Jubilee meeting of the American Chemical Society Sept. 4th, 1951..

or some retain their activity well, while others may lose a great part or even all of it. This hypothesis made it possible to investigate the problem experimentally because the nitrogen content of unicellular organisms can easily be reduced either by culturing them in solutions with very low nitrogen content or by suspending the cell mass with normal protein content in sugar solution under vigorous aeration. In the former way Virtanen and De Ley (2) have obtained from a strain called *B. coli*  $K_3$  — this strain is in reality an *Aerobacter* strain, as later was proved — cell masses with very different N-content, the extreme values being about 14 and 7 per cent N of dry matter. By using the same method Virtanen and Kokkola (3) have obtained bacterial masses from *Pseudomonas fluorescens*, the N-content of which has varied from 13.7 to 5.4 per cent of dry matter. By suspending *Torula utilis*-yeast in sugar solution under vigorous aeration the N-content of cells was lowered from nearly 10 to slightly below 5 per cent and by adding  $\beta$ -alanine even to 3.7 per cent [Virtanen and Aejmelaesus, (4)].

Before I proceed to record our results regarding the effect of the lowering of the N-content of cells on their enzymatic activity I must briefly mention some preliminary results concerning the question which N-fractions in the first place are affected by the decrease and how the N-content of the nutrient solution affects the growth and N-content of the cells.

The observations made with *Ps. fluorescens* (3) are illustrated by Fig. 1.

It appears from the figure that both the growth of bacteria and the N-content of cells reach their optimum in the same ammonium sulphate concentration, which in the nutrient solution used by us has been 2 g  $(\text{NH}_4)_2\text{SO}_4$  (0.4 g N) per 1 litre.

On both sides of this ammonium sulphate concentration the N-content of the cells gradually lowers, a steep fall occurring only at very low ammonium concentrations when the N-nutrition becomes rapidly consumed. The figure shows also that the N-fraction insoluble in trichloroacetic acid, to which the proteins belong, is greatest (about 85 per cent of total nitrogen) in cells with the highest N-content. It is interesting to note, that the insoluble N-fraction in per cent of total N is practically the same in bacterial cells grown in nutrient solution with superoptimal and lowest ammonium sulphate concentration. The results reveal distinctly that the

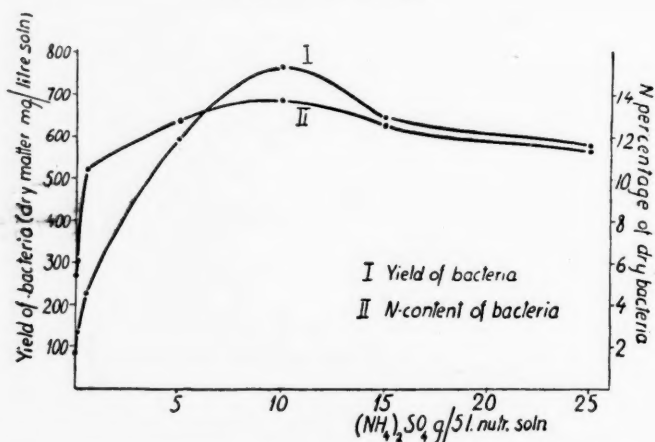


Fig. 1. — Dependence of the growth and N-content of *Ps. fluorescens* on the ammonium sulphate concentration of the nutrient solution.

$(\text{NH}_4)_2\text{SO}_4$ g 5 l. nutrient solution	N-content of bacteria. % of dry matter	N-fraction insoluble in trichloroacetic acid, % of total N in bacteria
25	11.5	76
15	11.8	81.8
10	12.4	85.9
5	11.9	84.3
0.5	10.1	77.2
0.1	6.1	76.4
0.05	5.4	75

TABLE 1

DIFFERENT NITROGEN FRACTIONS IN NORMAL AND LOW-NITROGEN MICROORGANISMS

g per 100 g dry matter	<i>Ps. fluorescens</i>			<i>Torulopsis utilis</i>		
	Normal N Bact. Mass	Low-N Bact. Mass	Decrease %	Normal N Yeast Mass	Low-N Yeast Mass	Decrease %
1. Total N . . . . .	12.7	7.8	38.6	9.5	5.7	40
2. «Protein N» . . . .	9.18	5.52	39.9	6.1	4.1	33
3. N soluble in tri- chloroacetic acid	3.50	2.30	34.3	3.4	1.6	53
4. Na-N . . . . .	2.27	1.05	54.7	1.4	0.65	53.5
5. Other soluble N	1.23	1.25	0	2.0	0.95	52.5

«Protein-N» = calc. difference between 1 and 3. Other soluble N = calc. difference between 3 and 4. Na-N=Nucleic acid N.

amount of nitrogen nutrition, affects not only the nitrogen content of the cells but also the mutual relations of different nitrogen fractions.

A more detailed picture of the changes in different N-fractions accompanying the lowering of the N-content of cells is given by Table 1, taken from the paper by Virtanen and Miettinen (5).

The results show that the nucleic acid fraction lowers more distinctly than the protein fraction both in *Pseudomonas fluorescens* and *Torula*. The changes occur entirely in the ribonucleic acids. Desoxyribonucleic acids, on the other hand, do not decrease while the N-content of the cell lowers, as De Ley has proved microscopically and Miettinen analytically. In *Torula* the protein fraction lowers less than the total N, in *Pseudomonas* vice versa. Hence, differences occur between different microorganisms with respect to the N-fractions while the N-content of the cells lowers. However, it can be said roughly that the changes in the total N also indicate changes in the protein-N.

When the enzymatic activity of normal and low-nitrogen cells was examined attention was fixed to the indispensable enzymes, which play a central part in the metabolism, and to the enzymes, which presumably are not indispensable except when the corresponding substrates form the sole energy source of the cells (such as lactase, saccharase, etc.). The latter are often adaptive or semi-adaptive, in other words, they are formed either in the presence of the substrate only or at least are activated enormously by the action of the substrate. Lactase is completely adaptive in the said *Aerogenes* K<sub>3</sub>, the activity of saccharase in saccharose-free nutrient solutions is about 10 per cent of that formed in saccharose nutrient solutions. Constitutive enzymes, i.e. those largely independent of the composition of the nutrition, are, for instance, catalase and the respiration enzyme system. The function of the catalase in the organism is not yet fully understood. It is indispensable to aerobic organisms but not to anaerobic ones.

The numerous experiments carried out together with De Ley (2) showed concordantly (Fig. 2) that as the N-content of the bacterial mass lowers, the saccharase activity immediately sharply decreases, being about 10 per cent of the corresponding activity of the normal cells when the N-content of the cells was lowered from 13 to 11 per cent, i.e. only 15 per cent. The catalase activity, instead, does

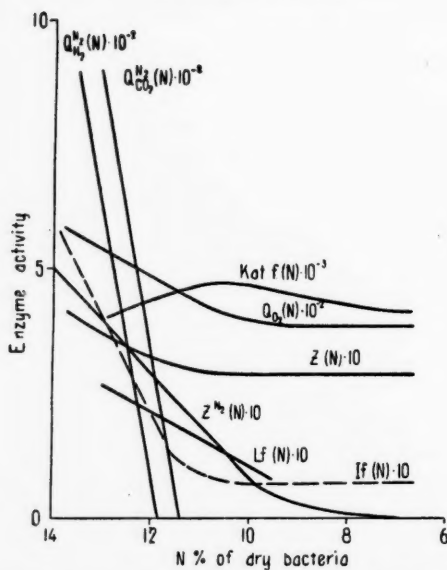


Fig. 2. — Changes in the activity of different enzymes as affected by the N-content of *Aerobacter*. All activities calculated per N-content of cells.

Kat. f. (N)  $\cdot 10^{-3}$  Catalase effect (Virtanen and De Ley); If (N)  $\cdot 10$  Saccharase effect (Virtanen and De Ley);  $Q_{O_2}(N) \cdot 10^{-2}$  Respiration (De Ley); Z (N)  $\cdot 10$  Aerobic acid formation (De Ley);  $Z^{N_2}(N) \cdot 10$  Anaerobic acid formation (De Ley);  $Q_{H_2}^N(N) \cdot 10^{-2}$   $H_2$  formation from formic acid (De Ley);  $Q_{CO_2}^N(N) \cdot 10^{-2}$   $CO_2$  formation from formic acid (De Ley); Lf (N)  $\cdot 10$  Lactase effect (Virtanen and Winkler).

not lower at all while the N-content of the cells falls from 13 to 7 per cent, or nearly 50 per cent. On the contrary, it rises even noticeably or in many experiments strongly while the N-content lowers as little as 15 per cent. These results already showed that the working hypothesis taken for the starting point is evidently fruitful. The completely adaptive lactase was later proved to behave as saccharase, about 10 per cent of its normal activity was preserved in low-nitrogen cells.

In his doctoral thesis De Ley (6) continued the research with special regard to the enzymes catalyzing the energy-giving metabolism reactions. His research disclosed that these enzymes are fairly well preserved in the low-nitrogen cells. The respiration rate of the cells containing only 7 per cent N, or about 50 per cent of the normal, is still about 70 per cent of the maximum. When saccharose, lactose, glucose, fructose, succinate, glycerate, pyruvate, and for-

mate were used for the substrate, the respiration rate diminished in N-deficiency as much with each of them. Thus it seems that the 10 per cent of saccharase and lactase, which remain in the low-nitrogen cells, suffice to hydrolyze the respective disaccharides at such a rate that they still can act as substrates in the energy-liberating reactions practically as well as the corresponding monosaccharides. From his results De Ley concluded that dehydrogenases, diaphorases, cytochromes and cytochromic oxydases are preserved in the N-deficient cell. The decrease of 30 per cent in the respiration rate of low-N bacteria as compared with normal ones should be attributed to the diminishing of the adaptive dehydrogenase proteins.

De Ley also made the interesting observation with *Aerobacter* K<sub>3</sub> that the formation of acids from glucose rapidly weakens in *anaerobic* conditions while the N-content of the cells lowers, ceasing completely in cells with a nitrogen content of about 8 per cent of dry weight. Respiration continues then still at a rate of 70 per cent of normal. Fermentation is thus prevented although respiration continues as does also formation of acids under *aerobic* conditions.

Further, it was shown that decarboxylating enzymes disappear from low-N cells and that adaptive enzymes which catalyze the formation of H<sub>2</sub> and bicarbonate from formate, disappear completely from the cells when the N-content is lowered only with 20 per cent.

Together with Aejmelaeus (4) I have examined the rate of respiration and fermentation of glucose and the activity of catalase and saccharase in *Torula* yeast, the N-content of which has varied between 10 and 3.7 per cent. In *Torula*, contrary to *Aerobacter*, saccharase is a constitutive enzyme, which is formed to approximately the same extent in the cells both when glucose and saccharose form the carbon source. It was therefore interesting to note how the lowering of the N-content affects the saccharase activity of *Torula*. The results appear from Fig. 3.

The curves show that the rate of respiration, catalase and saccharase activity, calculated per N-content of cells, rise somewhat while the N-content of the yeast drops from 10 per cent to about 5 per cent, or to half of the normal. A steep fall occurs only from this onward but yeast has then already reached the extreme limit of its living possibilities and the cell mass may already con-

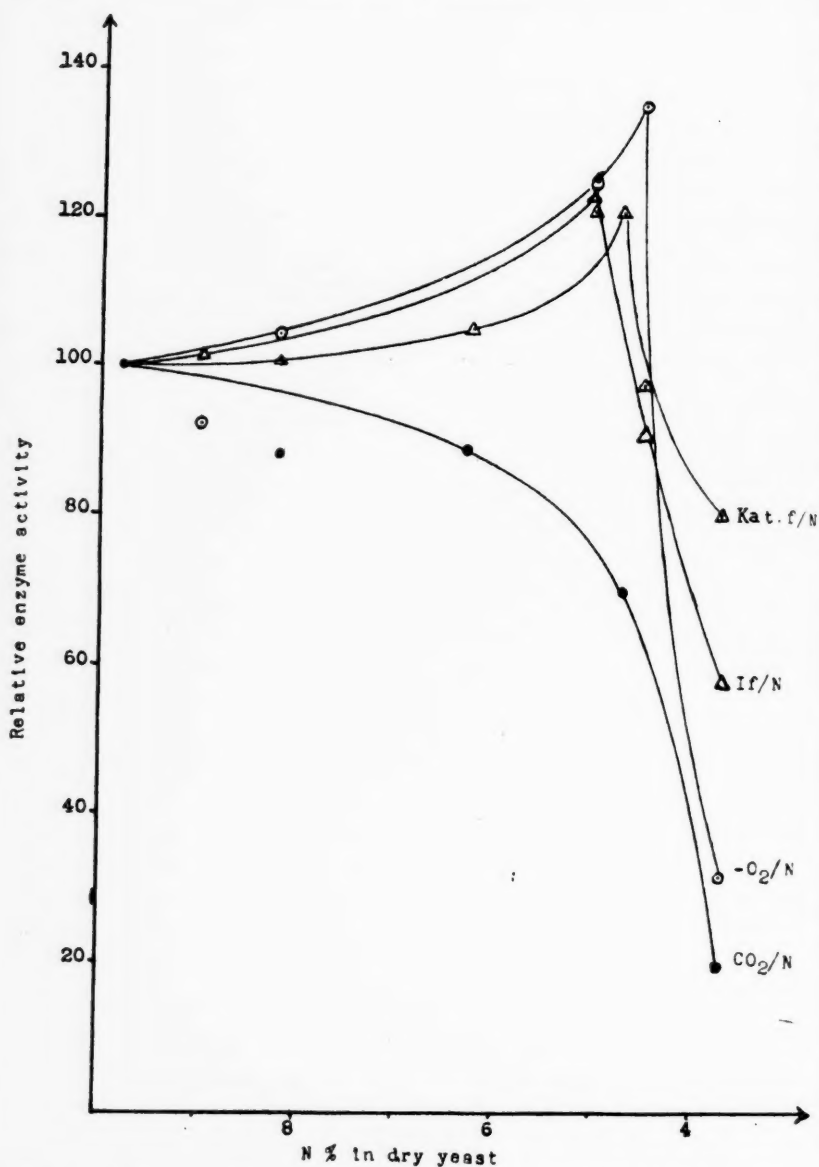


Fig. 3. — Experiments with *Torula utilis*. Influence of the N-content of cells on the consumption of oxygen under aerobic conditions, evolution of carbon dioxide under anaerobic conditions, and saccharase and catalase activities. Activity of normal-N cells marked with 100.

tain a great number of dead cells. Consequently, constitutive saccharase in yeast differs entirely from the adaptive saccharase in *Aerobacter* as regards dependence of this enzyme on the N-content of cells.

Fermentation lessens distinctly as the N-content of *Torula* diminishes. As the N-content is lowered to half, the rate of fermentation has fallen about 30 per cent. Fermentation did, however, not cease completely in *Torula* even at extremely low N-concentrations.

With *Pseudomonas fluorescens* I observed with Miss Kokkola (3) that respiration is well retained even if the N-content of the cells lowers from 13.7 to 5.4 per cent. Anaerobic fermentation decreases then considerably, thus the rate of fermentation decreases in all organisms examined as the N-content diminishes.

*Ps. fluorescens* excretes proteinase to the nutrient solution as was shown in this laboratory in the beginning of the 1930's (7). The excretion which explains the liquefying of gelatin by gelatin-liquefying bacteria is so quantitative that only a few percent of the total activity of proteinase is found in the bacterial mass separated by centrifugation and the rest is found in the clear nutrient solution. Peptidases, on the other hand, are retained quantitatively by the cells. In cultivating *Ps. fluorescens* in low-nitrogen and high-nitrogen nutrient solutions the activity of proteinase was in every case nearly the same although the N-content of the cells varied greatly.

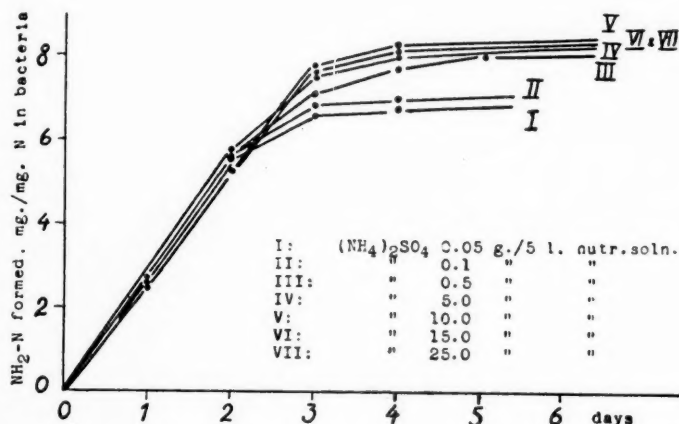


Fig. 4. — Influence of N-nutrition on the decomposition of casein by the excreted protease of *Pseudomonas fluorescens*.

In the investigations with *Aerobacter* K<sub>3</sub> carried out with Miss Winkler (8) we noted that the proteolytic activity of cells was fully retained while the N-content fell from 14 per cent to 10 per cent (Fig. 5). It seems that the proteolytic enzyme system as well as the

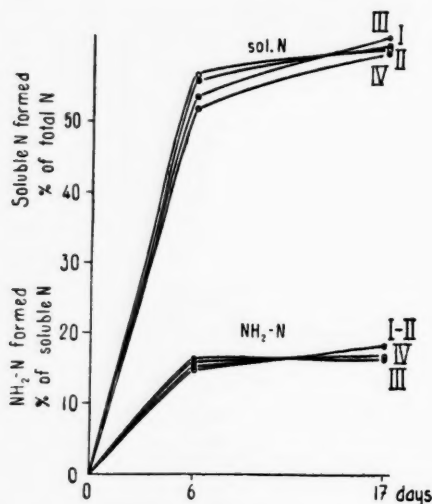


Fig. 5. — Formation of soluble and amino nitrogen from casein by *Aerobacter*

Curve	I: 13.2 % N of dry bacteria
»	II: 12.6 » » » » »
»	III: 10.4 » » » » »
»	IV: 10.4 » » » » »

respiration system are well preserved in the cells inspite of the decreasing N-content. It is quite natural if we presume the proteolytic enzymes to participate both in the hydrolysis of polypeptides and in their synthesis from lower peptides. The investigations made in the recent years in this laboratory on the enzymatic synthesis of polypeptides support this assumption (9).

As I already mentioned, the rate of fermentation diminishes in quite a different proportion than that of respiration as the N-content of the cells lowers. De Ley (6) observed with *Aerobacter* K<sub>3</sub> that acid formation entirely ceases in anaerobic conditions as the N-content of the cells falls to about 8 per cent. This observation is in many respects highly interesting. For the first, it seems to indicate that fermentation as an energy-giving process acts in this bacterium

only under good or at least moderate nutritional conditions and is thus comparable with adaptive enzyme systems. For the second, it raises the serious question whether the present idea of the similarity of the mechanisms of respiration and fermentation over triose phosphates is possibly too one-sided. If under anaerobic conditions no acids are formed from glucose with low-N bacteria as De Ley found, decomposition of sugar ought to cease at least at triose phosphate stage. Since the same low-N bacterial mass still respire, the respiration ought to differ from fermentation at least at this stage. Another possibility, discussed by De Ley, is that in the respiration process of low-N bacteria oxidation would proceed directly from glucose-6-phosphate to phosphogluconic acid. Oxidation of unphosphorylated glucose to gluconic acid might also come into question. Respiration and fermentation would thus differ from each other at the very start. For the present, there is no conclusive evidence for the opinion that low-N *Aerobacter* would respire glucose over phosphogluconate or gluconate. In any case lowering of the N-content of the cells seems to open new possibilities for studying the mutual relations of respiration and fermentation and their reaction mechanisms.

In the investigations with *Aerobacter* K<sub>3</sub> on the effect of the N-content of cells on the fermentation products of sugar the following results were obtained (10).

We could not lower the N-content to below 9 per cent. With bacterial masses containing a little over 9 per cent N, the rate of fermentation of glucose, calculated per nitrogen content of the cell mass, was lowered to about half of that with normal-N bacteria, *i.e.* much less than in the experiments by De Ley. The rate of fermentation was determined both on the basis of the sugar uptake and gas evolution. In the mutual relations of the fermentation

TABLE 2  
FERMENTATION PRODUCTS OF GLUCOSE WITH NORMAL- AND LOW-NITROGEN  
*Aerobacter*

No.	Normal-N Bacteria					Low-N Bacteria				
	Formic Acid	Ethanol	Lactic Acid	Acetic Acid	Succinic Acid	Formic Acid	Ethanol	Lactic Acid	Acetic Acid	Succinic Acid
4	6.5	12.4	40.0	1.5	29.3	5.5	—	21.1	13.9	39.6
5	5.6	12.6	41.8	2.7	28.0	4.6	11.0	21.0	10.5	37.8

products typical differences were noted between normal and low-N bacterial masses. pH and other experimental conditions were the same in both cases. Table 2 gives results of two experiments.

Thus lactic acid formed in the low-N bacteria is only about half of that in normal-N bacteria. On the contrary, acetic acid and succinic acid have increased in low-N bacteria. On the basis of the results it seems possible that the activity of lactic acid dehydrogenase in low-N bacteria has weakened, which affects the rate of hydrogenation of pyruvic acid. Relative strengthening of the reaction path A again leads to an increase in acetic acid and succinic acid. This explanation is only hypothetical until it has been shown, that the activity of lactic codehydrogenase is lowered in low-N cells (Fig. 6).

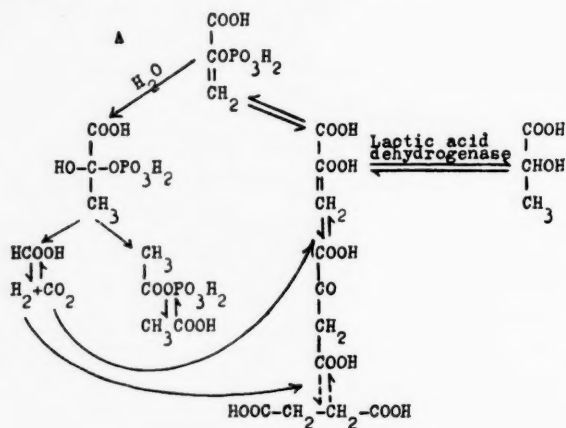


Fig. 6.

In this connection it should be mentioned that as early as in the beginning of the 1930's I noted with Dr. H. Karström (11) that in a nutrient solution, which contained very little ammonium sulphate, *E. coli* produced succinic acid twice the amount there was in a solution containing liberally nitrogen nutrition. We then explained this by assuming that when ammonium nitrogen was the minimum factor, the synthesis of aspartic acid from fumaric acid through aspartase was weakened and fumaric acid was reduced to succinic acid. Michaelis (12) examined later in detail this reaction in this laboratory. He regarded the result to be in agreement with the above assumption.

In the experiments we have now carried out with normal-N and low-N bacteria masses, fermentation solution did not contain any ammonium nitrogen, hence, the changes in the quantitative relations of the fermentation products are evidently due to the changes taken place in the enzyme system.

The observations I have reported reveal the very great effect of the lowering of the N-content of cells on the activity of different enzymes. The set working hypothesis of the high activity of indispensable enzymes in low-N cells and of the low activity of enzymes indispensable only under certain nutritional conditions has proved to hold good with the experimental results. The notable decrease of ribonucleic acid accompanying the lowering of N-content of cells may considerably effect on the protein, and accordingly, enzyme synthesis. The role of the nucleic acids of cytoplasm in the protein synthesis is still obscure but it seems that a powerful synthesis occurs in cytoplasm (1c). Spiegelman (13) has given special attention to the participation of cytoplasm in the enzyme formation.

Naturally one may also presume that the adaptive enzyme formation concerns formation of prosthetic groups and that the specific protein is always in the cell. In organisms, such as the bacteria of *Coli-aerogenes* group, which live on as simple a nutrition as possible, without any specific organic molecule groupings, N-deficiency may of course depress, not only the protein and nucleic acid synthesis, but also that of coenzymes. Decrease in the activity of certain enzymes would thus be natural. However, with many of the enzymes studied by us, as *e.g.* with saccharase and lactase, the lack of a specific protein is the most probable cause of the lessening or disappearance of enzyme activity. Spiegelman has some years ago showed that with yeast, which has been trained to use galactose, the question is of the formation of an apoenzyme, *i.e.* a specific protein. Monod (14) has recently proved conclusively that the adaptive formation of  $\beta$ -d-galactosidase in *E. coli* involves the synthesis of a specific protein.

In animal tests some investigators have been able to note the effect of the protein content of nutrition on the enzymatic activity of liver. Miller (15) observed in the liver of fasting rats after 7 days a decrease in the activity of some enzymes to be of the same degree as or higher than the protein loss of the tissue. Harkness *et al.* (16) found in the liver of rats receiving a protein-deficient diet a de-

crease in the concentration of co-enzyme A. In Bargoni's (17) recent experiments with protein-deficient rats, when the N-content of the liver lowered to 37 per cent of the normal, phosphomonoesterase showed a very remarkable increase, dipeptidase remained unchanged, pyrophosphatase, adenosintriphosphatase, arginase and succinic dehydrogenase were lowered in some degree and esterase, catalase and choline dehydrogenase very much. For the present there is no certainty of to what extent the lowering of the activity of different enzymes, which accompanies the decrease of the N-content of liver, is due to the decrease in enzyme proteins or co-factors. On the basis of the observations made on micro-organisms it can, however, be assumed that the protein loss may be in many cases a decisive factor even in liver.

The decisive role of the N-nutrition and consequently of the N-content of cells in the formation of enzymes attracts the fancy to the question which role the N-nutrition has probably had and still has in the origin of new species and forms. From the results recorded it can be concluded that while the N-content of the cells is optimal the best possibilities exist for the formation of enzymes. The adaptability of cells to nutritional conditions of greatest possible variety is then optimal. On the contrary, the organisms living on a very N-deficient nutrition need their protein for building up indispensable enzymes, hence, their adaptability is poor. It can thus be presumed that the abundance of nitrogen nutrition also increases the mutation possibilities of the species for acquiring new enzymes and hence, new properties.

Deficiency in many other elements besides nitrogen can influence the activity of the enzymes in the cell but the influence of nitrogen as an essential element in the protein molecule is evidently most general. In this connection the recent finding of Rautanen and Kärkkäinen (18) on the phosphorus content and acid phosphatase activity of *Torula* yeast is of interest. Rautanen observed that during phosphorus starvation the acid phosphatase activity rises enormously. When the low-phosphorus *Torula* takes up phosphorus the acid phosphatase activity rapidly decreases. Thus in this case the influence of phosphorus deficiency on the enzyme activity is contrary to that of N-deficiency in our experiments.

In conclusion, I still want to mention the following of the adaptive enzyme formation. The results suggest that an organism,

(17) which has an inherited ability to form certain enzyme adaptively, produces such an enzyme — at least in the cases examined by us — in all cells and not via mutation. As the concept is still fairly dominant that adaptation is a result of mutation and selection, I may mention one simple experiment on *Aerobacter* K<sub>3</sub> made with Winkler (4). In my opinion it speaks for the concept that practically all *Aerobacter* cells acquire through adaptation an ability to form lactase.

*Aerobacter* K<sub>3</sub> which was repeatedly cultivated in saccharose nutrient solution (90 times during 180 days) and which did not show any lactase activity was transferred to liquid lactose-agar nutrient (45° C). The lactose agar contained sufficiently bromcresol purple to colour the solution deep violet. Different dilutions of bacteria were made and the contents of the tubes were emptied into Petri dishes. After two days at room temperature, colonies were formed, which were encircled by a yellow zone because of acid formation from lactose (bromcresol purple changes to yellow at pH less than 6.2). In a parallel experiment with glucose-agar, approximately as many colonies were formed, and the colour of the indicator had also changed to yellow around all colonies. Table 3 records one of the experimental series.

TABLE 3  
NUMBER OF COLONIES (PER CM<sup>2</sup>) OF LACTASE-FREE *E. Coli* (K<sub>3</sub>) ON LACTOSE AND GLUCOSE-AGAR

	Dilutions				
	I	II	III	IV	V
Lactose-agar	Countless numbers	257	74	40	2.1
Glucose-agar	Countless numbers	281	92	44	2.6

This result is difficult to interpret by assuming that the lactase has been formed through mutation and selection. Formation of adaptive enzymes, on the other hand, as a result of training of long duration may be ascribable to mutation and selection.

#### REFERENCES

1. a) VIRTANEN, A. I.: Suomen Kemistilehti B, 1942: 15: 22; see also b) VIRTANEN, A. I.: Sitz. Ber. finn. Akad. Wiss. 1944; 1945: 131; and c) VIRTANEN, A. I.: Svensk Kem. Tidskr. 1948: 60: 23.
- 17 — Ann. Med. Exper. Fenn. Vol. 30. Fasc. 3-4.

2. VIRTANEN, A. I., and DE LEY, J.: *Arch. Biochem.* 1948: 16: 169;  
VIRTANEN, A. I.: *Fourth Int. Congr. Microbiol. Copenhagen 1947*,  
*Report of Proc.* 1949: 379.
  3. VIRTANEN, A. I., and KOKKOLA, U.: *Acta Chem. Scand.* 1950: 4: 64.
  4. VIRTANEN, A. I.: *Colloid Chem.* 1950: 7: 255.
  5. VIRTANEN, A. I., and MIETTINEN, J. K.: *Acta Chem. Scand.* 1949 3:  
1437.
  6. DE LEY, J.: *Over de Fermenten van Stikstof-Arme Bacterium Coli.*  
*Diss., Verhandel. Koninkl. Vlaam. Acad. Geneesk. Belg.* 1949: 11:  
no. 3.
  7. VIRTANEN, A. I., and TARNANEN, J.: *Naturwissenschaften* 1931: 19: 397;  
*Ztschr. physiol. Chem.* 1932: 204: 247; VIRTANEN, A. I., and SUO-  
LAHTI, O.: *Enzymologia* 1937: 2: 89 and 1937: 3: 62.
  8. VIRTANEN, A. I., and WINKLER, U.: *Acta Chem. Scand.* 1949: 3: 272.
  9. VIRTANEN, A. I., KERKKONEN, H. K., LAAKSONEN, T., and HA-  
KALA, M.: *Acta Chem. Scand.* 1949: 3: 520; VIRTANEN, A. I., KERK-  
KONEN, H. K., HAKALA, M., and LAAKSONEN, T.: *Naturwissenschaften*  
1950: 37: 139; VIRTANEN, A. I.: *Makromolekuläre Chemie* 1951: 6:  
94; VIRTANEN, A. I.: *Rend. ist. super. sanità* 1950: 13: 894.
  10. VIRTANEN, A. I., and ALONEN, S.: *Acta Chem. Scand.* 1952: 6: 654.
  11. VIRTANEN, A. I., and TARNANEN, J.: *Biochem. Ztschr* 1932: 250: 1 f 3,  
p. 197.
  12. MICHAELIS, M.: *Ztschr. physiol. Chem.* 1935: 237: 181.
  13. SPIEGELMAN, S.: *Arch. Biochem.* 1947: 13: 113; *Cold Spring Harbor*  
*Symposia Quant. Biol.* 1947: 11: 256.
  14. MONOD, J.: *Abstr. Am. Chem. Soc. 120th Meeting* 1951: 2 A.
  15. MILLER, L.: *J. Biol. Chem.* 1948: 172: 113.
  16. HARKNESS, D. M., SEIFTER, S., NOVIC, B., and MUNTWYLER, E.:  
*Arch. Biochem.* 1949: 22: 204.
  17. BARGONI, N.: *Experientia* 1951: 7: 104.
  18. RAUTANEN, N., and KÄRKKÄINEN, V.: *Acta Chem. Scand.* 1951: 5: 981.
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# PROTEIN DETERMINATION IN SPINAL FLUID AND URINE BY *pH* INDICATOR METHOD

by

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(Received for publication April 4, 1952)

There has been mention of the disturbing effect of protein on *pH* indicators ever since 1909 (Sørensen, 6) in the scientific literature. Feigl and Anger (2) and Ishidate and Sakaguchi (3) used this phenomenon to detect proteins qualitatively. The latter investigators tested 27 *pH* indicators for this purpose and found 10 sensitive to protein, the most sensitive being the potassium salt of tetrabromophenolphthalein ethyl ester. Joukovsky and Vandervelden (4) lectured about the estimation of protein concentration in blood serum by means of this indicator. In order to select the best indicator for protein determination the authors of this paper tested 14 other *pH* indicators and reaffirmed that the potassium salt of tetrabromophenolphthalein ethyl ester is the most suitable for protein determination.

The potassium salt of tetrabromophenolphthalein ethyl ester was synthesized according to Nietzki and Burckhardt's procedure (5). This salt is blue and in solution its colour changes from blue through green to yellow in the *pH* region 5.5—4.5. Feigl (1) says that this indicator reacts only with native proteins, not with amino acids, di- and tripeptides or peptones. The protein compound of tetrabromophenolphthalein ethyl ester remains unbroken and therefore blue on the acid side of *pH* 4.5 (in dilute acetic acid solutions). Determination of proteins must therefore be done in solution under this *pH* value. The shade of the colour and the clearness of the analysis solution depends on the preparation order.

## EXPERIMENTS

*Indicator Solution.* — 0.05 per cent (w/v) tetrabromophenolphthalein ethyl ester potassium salt in ethyl alcohol.

*Buffer solution.* — 5.0 ml of 0.1 N acetic acid and 95.0 ml of 0.1 N sodium acetate (pH 3.4). The buffer capacity of this solution was sufficient to keep the pH value of urine dilution used in this method on the acid side of pH 4.5, although pH of urine was made to 8.6 with phosphate buffer.

*Procedure.* — To 0.5 ml of *spinal fluid* 9.5 ml of water and one ml of buffer solution and after mixing well one ml of indicator is added. To 10 ml of *urine* dilution with water 1 : 100 one ml of buffer and one ml of indicator solution is added. Reading in photometer must be done after 10 but before 30 minutes using as reference solution a mixture of 10 ml water, one ml buffer and one ml indicator solution. The wavelength of maximum light absorption  $610 \mu$  was measured with Beckman spectrophotometer. The protein content of the unknown spinal fluid or urine may be read from the calibration curve constructed with pure bovine albumin (Fig. 1.)

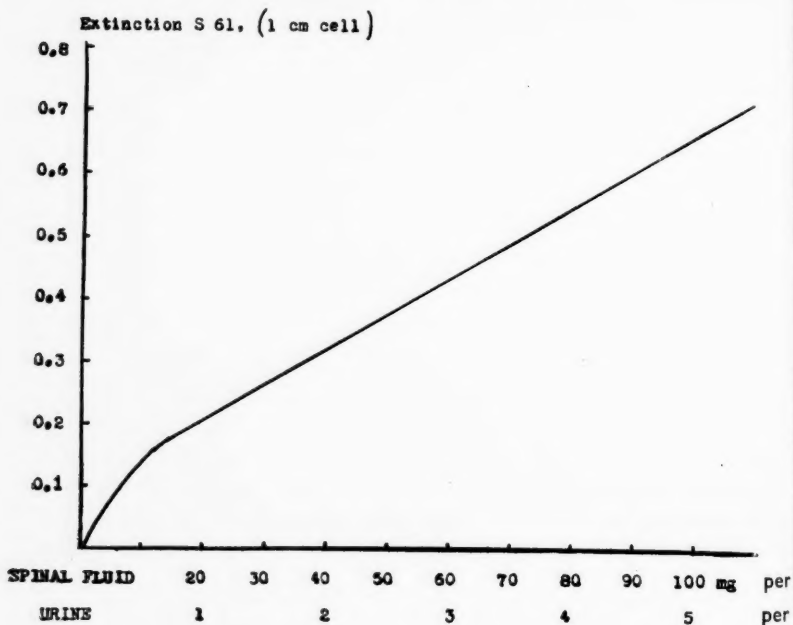


Fig. 1. — Standard curve with Pulfrich stufenphotometer.

If the spinal fluid contains more than 100 mg per 100 ml and the urine more than 5 pro mil protein the sample must be further diluted. If the protein concentration is small the urine sample may be diluted less than 100 times. When the urine is diluted 1 : 100 its colour has no effect in photometer reading, but if the dilution is less than 1 : 100 the possible error may be corrected by comparing the urine dilution with water. The larger creatinine content in urine may have some effect on results if the dilution is less.

With this method it is also possible to estimate the concentration of other native proteins by diluting suitably.

The protein content of two spinal fluid mixtures was estimated with micro-Kjeldahl method. The results were 42 mg per 100 ml and 56 mg per 100 ml. The indicator method gave 40 mg per 100 ml and 57 mg per 100 ml.

The protein concentration of pathological urine obtained by the indicator method was compared with results obtained by the biuret method, where protein was precipitated with trichloroacetic acid (Table 1).

TABLE 1

PROTEIN CONTENTS PER MIL IN A SERIES OF PATHOLOGICAL URINES BY BIURET AND INDICATOR METHOD

Biuret	Indicator
1.2	1.3
2.5	2.6
1.7	1.7
2.3	2.4
5.6	5.6
1.7	1.6
13.7	13.2
4.1	4.0
3.1	3.3
3.5	3.7

In order to find out in what manner different native proteins react, dilution series of a known blood serum, bovine albumin and spinal fluid were measured with Beckman spectrophotometer. The results are recorded in Fig. 2. It shows every protein type has the same effect on indicator.

The indicator concentration 0.1 (w/v) per cent in ethyl alcohol may be better than 0.05 per cent for urine protein determination because then the estimation region is larger. But this concentration is not suitable for spinal fluid, because a less dilution is used there. The increased salt content is inclined to precipitate the indicator. In

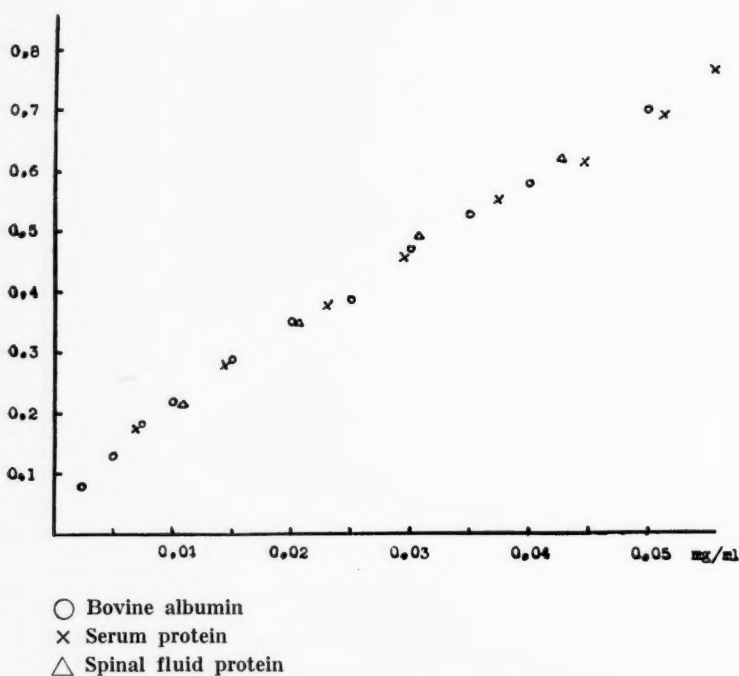


Fig. 2. — Extinction with the Beckman spectrophotometer DU.

order to prevent the precipitation different protect colloids were tested, but all of them seemed to have some effects on the colour of indicator solution. But 0.05 per cent indicator used in this method did not become precipitated during the measuring time.

In comparison with other methods for protein estimation in clinical use the advantages of this method are as follows: simplicity, rapidity, relatively great accuracy (the errors have remained under 10 per cent), small amount of the sample, neither corrosive acids nor alkalis have been used as reagents.

#### SUMMARY

A practical method for routine determination of protein in spinal fluid and urine is described in this paper. It is based on the native protein effect on the colour of a pH indicator, the potassium salt of tetrabromophenolphthalein ethyl ester in acid reaction.

# REFERENCES

- 1 FEIGL, F.: Qualitative Analysis by Spot Tests. New. York, 1947.
  - 2 FEIGL, F., and ANGER, V.: Microchim. 1937:2:107.
  - 3 ISHIDATE, M., and SAKAGUCHI, T.: Ber. Deutsch. Chem. Gesellsch. 1941: 74:163.
  - 4 JOUKOVSKY, N. I., and VANDERVELDEN, R.: XVIII International Physiological Congress, Copenhagen 1950:282.
  - 5 NIETZKI, R., and BURCKHARDT, E. D.: Ber. Deutsch. Chem. Ges. 1897: 30:175.
  - 6 SÖRENSEN, S. P. L.: Biochem. Ztschr. 1909:21:131.
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## BLOOD COMPOSITION OF HORSES BEFORE DEATH FROM CONTAGIOUS BRONCHOPNEUMONIA

by

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(Received for publication April 10, 1952)

The results of a previous investigation on contagious bronchopneumonia in horses showed that certain changes in the blood composition were typical of this disease (4). Altogether 25 horses in various stages of manifest infection were observed and blood values were obtained before and after the attack. In 9 cases the infection ended fatally. If the infection only appeared as a slight fever of one day's duration, no typical changes were detected in the blood composition, but if there were symptoms from the lungs and the fever lasted 3—4 days or more, certain changes in the blood composition occurred. A characteristic of the response was a decrease in inorganic phosphorus and vitamin A in the serum during the attack, followed by an increase during the stage of resistance. No changes were obtained in the calcium and ascorbic acid contents of the serum or in the nicotinic acid level of the blood. The percentage of eosinophils also decreased during the attack of bronchopneumonia, and an increase was noticeable later. In fatal cases the changes in the inorganic phosphorus and vitamin A values were not typical, whereas an eosinopenia appeared during the manifestation of the infection and in many cases lasted for a long time.

The investigation referred to above did not deal with variations in the blood composition during the stage of exhaustion. Facts concerning this stage of contagious bronchopneumonia are there-

fore reviewed here. It was seen that calcium, ascorbic acid in the serum, and nicotinic acid in the blood did not undergo change during the last two weeks before death, these values having changed only in one case out of six. Calcium was 10.6—12.9 mg per 100 ml, ascorbic acid 0.30—0.58 mg per 100 ml, and nicotinic 0.33—0.54 mg per 100 ml. The values obtained for inorganic phosphorus, vitamin A, and total protein are seen in Table 1.

TABLE 1

INORGANIC PHOSPHORUS (MG/100 ML), VITAMIN A (I.U./100 ML), AND TOTAL PROTEIN (PER CENT) IN THE SERUM OF HORSES IN FATAL CASES OF CONTAGIOUS BRONCHOPNEUMONIA

	During Attack	Two Weeks before Death	One Week before Death	Number of Horses
Inorganic phosphorus	$2.3 \pm 0.15$	$1.7 \pm 0.15$	$2.5 \pm 0.15$	5
Vitamin A .....	$101 \pm 10.3$	$62 \pm 10.3$	$56 \pm 10.3$	5
Total protein .....	$8.3 \pm 0.2$	$7.1 \pm 0.2$	$7.2 \pm 0.2$	6

When the data in Table 1 are examined, a drop will be seen in the values obtained for all three components listed. A thorough statistical analysis must be made, however, before any conclusions regarding typical changes can be drawn. There was no continuous decrease in inorganic phosphorus or total protein in the serum ( $0.1 < P < 0.2$ ), but a fall was noticeable in these components when the pneumonia increased in severity. This statement is certain, as it was significant at the 2 per cent level of probability for inorganic phosphorus and at the 5 per cent level of probability for total protein, according to Burn *et al.* (1).

The decrease might be due to other factors besides a depletion from the bones as the phosphorus and calcium contents in the femur were found to be normal, being 12.6—15.2 per cent  $P_2O_5$  and 17.5—19.7 per cent CaO.

A continuous decrease in the vitamin A content of the serum before death was significant at the 5 per cent level of probability when the pneumonia was aggravated. The decrease is considered a results of an inability to mobilize vitamin A, as the liver still contained more than sufficient amounts of this vitamin, i.e.,

60,000—140,000 I.U. per 100 g. According to Simola, the average content in normal horse liver is 68,210 I.U. per 100 g (3).

In the blood count the number of leucocytes remained on the same level for some weeks before death, with a slight increase in some cases during the last week (from  $13,000 \pm 0.140$  to  $14,000 \pm 0.140$  per cu mm). At the end, just before death, there was mostly a leucopenia, but leucocytosis did appear in some cases. In the relative eosinophil count a drop from  $7 \pm 1.6$  per cent to  $2 \pm 0.7$  per cent occurred in the last period before death. This fall is significant at the 2 per cent level of probability.

It has been a matter of doubt whether the blood groups are of importance when blood transfusions are given to horses. With regard to the horse the blood groups have been examined by Lehnert (2). The effect of blood transfusions as a treatment for emaciation in horses was tested in this study. Blood was taken from a horse of one blood group and given every second day to an emaciated horse of another group altogether three times. The horse receiving the blood died on the 9th day after the first transfusion.

A comparison of changes during the exhaustion caused by contagious bronchopneumonia and those which appeared during exhaustion due to repeated blood transfusions from another blood group will be seen from Table 2. Blood samples were taken in both cases every third day until death.

Similar changes in the blood composition seem to be present whether exhaustion is considered to be due to an allergic condition, repeated blood transfusions, or the action of some other stress, such as a bronchopneumonic infection. A decrease in inorganic phosphorus, vitamin A in the serum, and the eosinophil count in the blood are non-specific changes and appear when the organism is no longer able to respond effectively to the stressor. It was not, however, the absence of the above factors which was responsible for the drop in the blood values. It might be emphasized that the vitamin A values are about 10 per cent too low and the ascorbic acid about 40 per cent too low owing to a decrease in the values occurring during storage of the serum for 24 hours before the analyses were made.

From the point of view of prognosis a decrease in inorganic phosphorus, vitamin A, and the eosinophils, as well as a neutro-

TABLE 2

BLOOD COMPOSITION IN HORSES DYING FROM CONTAGIOUS BRONCHOPNEUMONIA  
(GROUP A) OR UNSUITABLE BLOOD TRANSFUSIONS (GROUP B)

	9 Days before Death		6 Days before Death		3 Days before Death		At Death	
	A	B	A	B	A	B	A	B
<i>Component:</i>								
Inorganic phosphorus (mg/100 ml serum) .....	3.3	3.0	0.8	2.3	0.8	2.9	0.9	1.6
Calcium (mg/100 ml serum) .....	12.2	13.1	11.0	12.0	11.5	13.0	10.6	12.5
Vitamin A (I.U./100 ml serum) .....	95.1	103.1	36.3	69.7	<35.0	72.6	<15.0	<10.0
Ascorbic acid (mg/100 ml serum) .....	0.39	0.48	0.46	0.51	0.32	0.40	0.30	0.43
Nicotinic acid (mg/100 ml whole blood) ..	0.40	0.53	0.41	0.47	0.54	0.46	0.54	0.45
Total protein (per cent in serum) .....	9.6	7.4	6.2	8.4	6.1	7.0	7.2	7.0
Erythrocytes (mill/cu mm) ..	6.8	8.4	7.6	5.6	7.6	6.2	—	5.4
Leucocytes (1000/cu mm) ..	7.9	13.8	7.3	11.3	22.4	10.9	—	18.0
Neutrophils (per cent) ....	76	53	70	70	97	62	—	79
Eosinophils (per cent) ....	4	2	10	5	0	8	—	1

philic hyperleucocytosis, may be regarded as unfavourable symptoms in horses.

## SUMMARY

In horses suffering from contagious bronchopneumonia a decrease occurred in inorganic phosphorus, vitamin A, total protein in serum, and eosinophil count in the blood during the stage of

exhaustion. The decrease in vitamin A was continuous. In calcium, ascorbic acid in serum, nicotinic acid, as well as in erythrocytes in the blood, no definite change could be detected. In some cases the eosinophils had totally disappeared and only traces of vitamin A were found at the time of death. These changes did not pertain to bronchopneumonia in horses only and may therefore be considered non-specific.

#### LITERATURE

1. BURN, J. H., FINNEY, D. J., and GOODWIN, L. G.: Biological Standardization. London 1950.
  2. LEHNERT, E.: Skandinav. Vet. Tidskr. 1934:24:401.
  3. SIMOLA, P. E.: Duodecim 1942:387.
  4. WESTERMARCK, H.: Studies on the Condition and the Blood Composition of Emaciated Horses Treated with Certain Nutritional Supplements during Recovery and during Contagious Bronchopneumonia. Academic Thesis 1952.
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## VARIATIONS OF SERUM COPPER IN CANCER OF THE UTERUS

by

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(Received for publication May 7, 1952)

The appearance of copper as a trace element in the organism, as well as its effect on enzymatic reactions of the cell metabolism has prompted several investigators to study the copper metabolism of the organism also in relation to malignant tumours. Edlbacher and Gerlach (2) found that the necrotic parts of malignant tumours contain an abundant amount of copper, although the copper content of the tumours is exceedingly variable. According to Sümegi (7), the tissue of malignant tumours is profuse in copper, and animals affected with tumours also reveal unmistakable changes in the copper content of various organs. Sugai (6) found that the copper level of malignant tumours was lower than in normal tissue or in benign cervical growths. On the other hand, in breast cancer the copper content of the tumour tissue somewhat surpassed that of normal tissue. Carruthers and Suntzeff (1) were able to demonstrate that transplantable squamous cell carcinoma in the rat is poorer in copper than normal epidermis.

Sachs, Levin and Fabian (5) found that the copper level in human whole blood was higher than normal in carcinoma patients, and similar results were obtained by Heilmeyer, Keiderling and Stüwe (4) in regard to serum copper. Greenstein and Thompson (3), on the other hand, found less copper than normal in both blood and serum of rats affected with Jensen's sarcoma.

Thus, unmistakable changes occur in the copper metabolism of the organism in connection with malignant tumours, and it has been generally assumed that the changes are due to the defense reaction of the organism. As a consequence, we began to study the copper metabolism in carcinoma patients, and have followed up the subsequent fate of the patients for a period of minimum four years, in order to throw light on the problem whether any difference in copper metabolism can be seen during radiation treatment between cases with a favourable and a poor prognosis.

*Material.* — Our series comprised 18 collum carcinoma and one corpus carcinoma cases treated at the X-ray Department of Women's Clinic. The patients were subjected to radium and x-ray therapy in accordance with the general therapeutic principles of the Clinic, and we have performed a total of 80 serum copper determinations for these patients in various stages of the treatment by radiation. The subsequent fate of the patients was followed up by means of questionnaires and examinations over a period of four years, and this fate has been clarified in all these cases, either by a visit from the patient, or with the aid of information obtained from the family or from the Register officials. Since the majority of the patients who died did so either at home or in the hospitals of their home communities, autopsies could not be performed. However, according to information obtained, all the deaths could be ascribed to carcinoma, as their direct or indirect cause.

*Method.* — In our studies of the copper metabolism of the organism the variations of serum copper were used as a standard of determination. For determining the serum copper the analytic method described by Heilmeyer, Keiderling and Stüwe was used, which is based on Callan and Henderson's sodium diethyl dithiocarbamate reactions and which, in addition to the original report, has also been described e.g. by Wallgren (8) in his article in *Nordisk Medicin*. The method proved to be reliable and sensitive.

*Results.* — Heilmeyer, Keiderling and Stüwe (4) have studied the serum copper content of 24 carcinoma patients, 5 of whom were affected with carcinoma of the genitals. In these latter cases the values of the serum copper contents varied between 150 and 239  $\gamma$  per cent, and all carcinoma patients had serum copper contents surpassing the normal. Table 1 presents the levels of serum copper which the patients of our series had on admission. When studying

TABLE 1

No	Diagnosis	Age, Years	Hb % (Sahli)	SR, mm	Serum Fe, $\gamma$ %	Serum Cu, $\gamma$ %	Follow-ups
1	Ca colli ut. gr. II ..... PAD: Ca basocellulare	41	75	68	15	170	Died 2 years later
2	Ca colli uteri gr. I ..... PAD: Ca	57	64	10	45	205	Living 4 years later
3	Ca colli ut. gr. II ..... PAD: Ca adenomatosum ..	39	63	16	80	165	Living 5 years later
4	Ca colli ut. gr. II ..... PAD: Ca	78	76	35	80	230	Died 2 years later
5	Ca colli ut. gr. IV ..... PAD: Ca basocellulare	52	65	46	60	165	Died 1 year later
6	Ca colli ut. gr. II ..... PAD: Adenocarcinoma	55	67	57	60	220	Died 2 years later
7	Ca colli ut. gr. IV ..... PAD: Adenocarcinoma	57	78	13	35	150	Died 9 months later
8	Ca corporis uteri ..... PAD: Adenocarcinoma .....	34	58	42	55	210	Living 4 years later
9	Ca colli ut. gr. I ..... PAD: Ca spinocellulare	28	68	6	60	165	Living 4 years later
10	Ca colli ut. gr. II ..... PAD: Ca ceratinosum	41	64	9	75	150	Died 2 years later
11	Ca colli ut. gr. II ..... PAD: Ca baso et spinocellulare	39	68	10	80	165	Died 2 years later
12	Ca colli ut. gr. III ..... PAD: Ca spinocellulare	47	50	27	35	140	Died 2 years later
13	Ca colli ut. gr. III ..... PAD: Ca basocellulare	45	69	12	65	160	Died 1 year later
14	Ca colli ut. gr. II ..... PAD: Ca spinocellulare	53	68	3	40	200	Died 1 year later
15	Ca colli ut. gr. III ..... PAD: Ca spinocellulare	51	73	46	50	160	Living 4 years later
16	Ca portionis gr. II..... PAD: Ca spinocellulare	38	64	32	65	185	Died 2 years later
17	Ca colli ut. gr. II ..... PAD: Ca ceratinosum	57	78	12	65	165	Living 4 years later
18	Ca colli ut. gr. III ..... PAD: Ca				70	185	Died 7 months later
19	Ca colli ut. gr. III ..... PAD: Ca				60	185	Died 5 months later

the table, we see that the serum copper content of patients with carcinoma of the uterus shows a marked rise, but the extent of the rise does not appear to be in accordance with the wideness of distribution of carcinoma in the organism, as the highest values were found in the first and second degree of collum carcinoma cases.

*According to Wallgren (8), the serum copper level of Finnish women has a range of  $120 \pm 15 \gamma$  per cent, whereas the levels presented on Table 1 show average value of  $178 \pm 6 \gamma$  per cent for serum copper contents of patients with uterine carcinoma. Since the classification of collum carcinoma into different stages according to the spread of the disease, performed on the basis of clinical examinations, is naturally inaccurate, the diagnosis of small metastases encountering insurmountable difficulties, we have found it advisable to base our investigations of the malignancy of carcinoma on the fate of the patient after the treatment. With this aim in view, we followed the fate of these patients over a period of four years, and the information we were able to collect is entered on Table 1. When the time of observation expired, there were 6 survivals in our series, the remaining 13 having died at various periods after the treatment. The average value of the serum copper content of the patients who survived was  $178 \gamma$  per cent and of those who died during the time of observation likewise  $178 \gamma$  per cent. No tendency towards lower or higher values is discernible in the serum copper contents of these different groups, both of them showing exceedingly high levels surpassing  $200 \gamma$  per cent as well as values only slightly in excess of the normal.*

*The Effect of Radiation Therapy on Serum Copper Contents. —* If we regard the serum copper content as an indication of the protective power of the organism against malignant tumours, it might be expected that some conclusions as to the development of the disease could be drawn from the variations of the serum copper level during radium treatment. For this purpose we have performed serum copper determinations on our patients at different stages of the radium therapy. Table 2 presents the results of these determinations obtained within the period of observation from the survived patients, while Table 3 shows the values of those patients who died during the time of observation. It is evident from these tables that *the serum copper level of both groups persists high with considerable*

TABLE 2

THE SERUM COPPER LEVELS OF SURVIVED PATIENTS DURING RADIUM TREATMENT

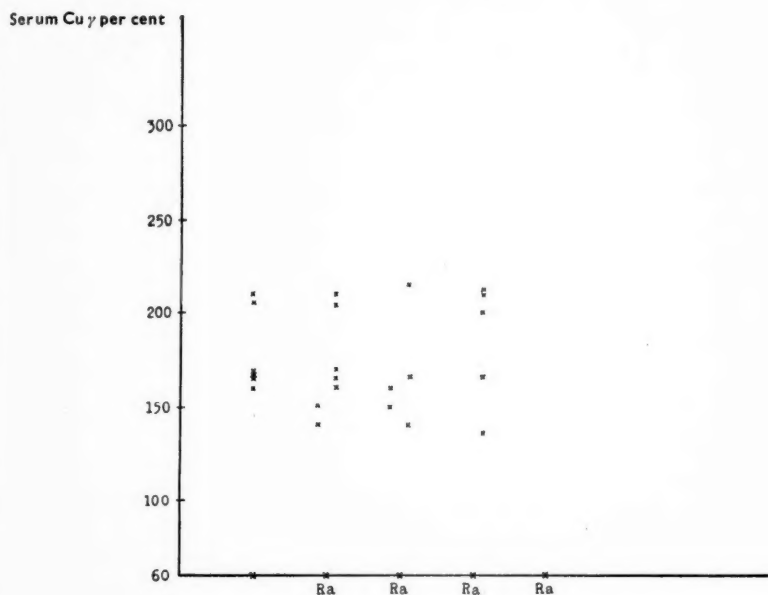


TABLE 3

THE SERUM COPPER LEVELS OF PATIENTS WHO DIED DURING THE TIME OF OBSERVATION, DURING RADIUM TREATMENT

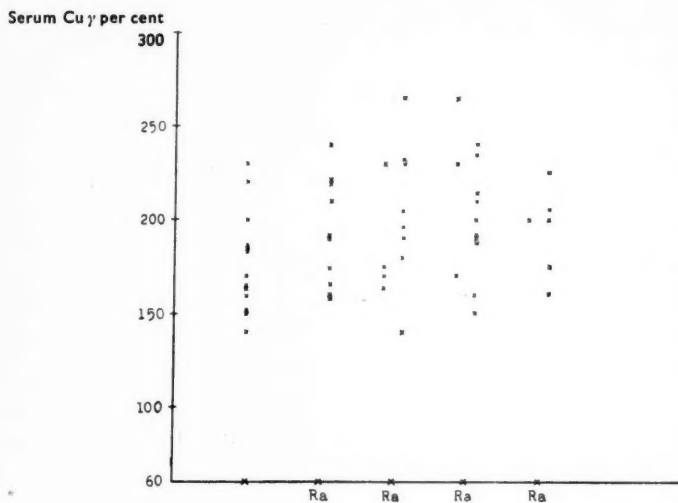
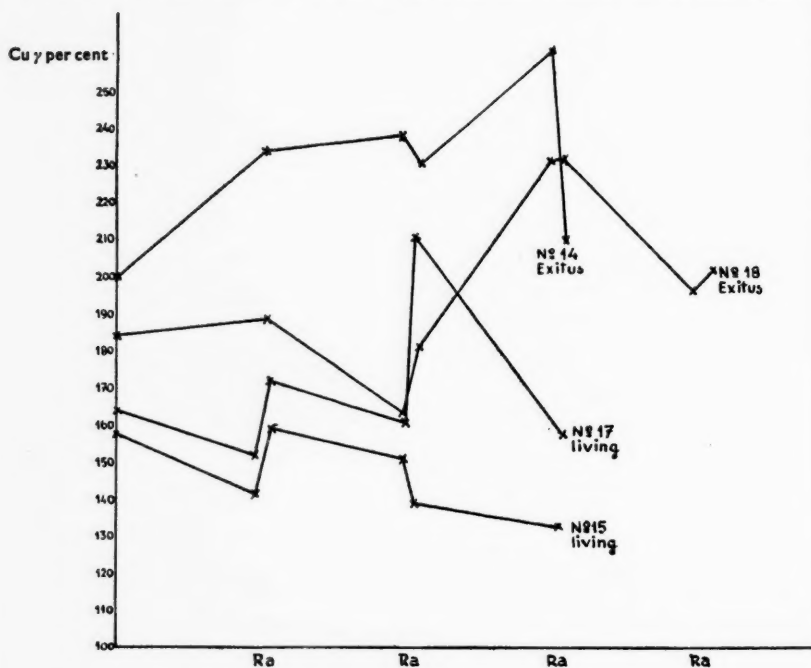


TABLE 4

SOME INDIVIDUAL CURVES OF SERUM COPPER LEVELS DURING RADIUM TREATMENT



regularity, nor can any difference be noted between recoveries and deaths. Table 4 presents some typical individual curves in both groups.

*Determination of Serum Copper in the Diagnosis of Gynaecological Growths.*—We have also studied the possibilities of using determinations of serum copper as an auxiliary method for diagnosing whether the gynaecological tumour is of a benign or malignant nature. With this aim in view 20 patients who were to be operated on for gynaecological growths were subjected to determinations of their serum copper prior to the operation. As shown by Table 5, *ten patients affected with benign tumours, or about half of the persons examined, revealed serum copper levels exceeding 140 γ per cent* (lowest value found in patients who suffered from malignant tumours), average value  $148 \pm 6$  γ per cent. High serum copper levels were especially found in those patients who had symptoms indicating necrosis or inflammatory processes. *Conse-*

TABLE 5

No	Diagnosis	Serum Cu γ per cent
20	Myoma uteri necroticans .....	190
21	Cystadenoma serosum carcinomatosum .....	175
22	Myoma uteri .....	165
23	Myoma uteri necroticans .....	155
24	Myoma uteri .....	150
26	Myoma uteri .....	140
27	Myoma uteri .....	140
28	Myoma uteri .....	175
29	Myoma uteri. Hydrosalpinx.....	190
31	Cysta dermoidea. Sactosalpinx. ....	115
32	Myomata uteri .....	145
33	Cysta ovari serosum. Torsio stili. ....	180
34	Myomata uteri .....	160
35	Myomata uteri .....	115
36	Myomata uteri .....	120
38	Myomata uteri .....	130
39	Myomata uteri .....	105
40	Tumor ov. sin. Cysta retentionis. Grav.m. II.....	140
41	Myomata uteri. Ulcus duodeni. ....	140
42	Cysta ov. sin.....	120

quently, the occurrence of high serum copper values in patients suffering from gynaecological tumours is no evidence of the existence of a malignant neoplasm.

*Conclusions.* — There is a rise of serum copper levels in cases of carcinoma of the genitals (in normal material  $120 \pm 15$  γ per cent, in carcinoma of the uterus  $178 \pm 6$  γ per cent).

No conclusions regarding a tendency to improvement or further advance of the cancer can be drawn from the extent of elevation of serum copper or its variations during radium treatment.

Increased serum copper values are also encountered in association with benign genital neoplasms.

#### REFERENCES

1. CARRUTHERS, C., and SUNTZEFF, V.: *Canc. Res.* 1945: 159: 647.
2. EDLBACHER, S., and GERLACH, W.: *Ztschr. Krebsforsch.* 1935: 42: 272.
3. GREENSTEIN, JESSE, P., and THOMPSON, J. W.: *J. Nat. Canc. Inst.* 1943: 3: 405.

4. HEILMEYER, L., HEIDERLING, W., and STÜWE, G.: Kupfer und Eisen als körpereigene Wirkstoffe. Jena 1941.
  5. SACHS, LEVIN, and FABIAN: Arch. Int. Med. 1935: 1: 227.
  6. SUGAI, M.: Mitt. med. Acad. Kyoto, 1940: 29: 314.
  7. SÜMEGI, S.: Frankf. Z. Path. 1935: 48: 35.
  8. WALLGREN, G. R.: Nord. Med. 1949: 41: 985.
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## ON THE ACTION OF HYALURONIDASE AND ITS INHIBITOR

by

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(Received for publication May 9, 1952)

The purpose of this paper is to draw attention to anomalies in the turbidimetric results obtained in the determination of hyaluronidase. These methods are widely used also in the estimation of concentration and degree of polymerisation of hyaluronate preparations. The anomalies referred to were seen in tests which were performed in order to elucidate the nature of the linkage between hyaluronidase and its serum inhibitor.

Although the turbidimetric methods have gained a wide use in the study of hyaluronate degradation, the kinetics of this reaction have not been thoroughly investigated. The work of Dorfman (2) reveals that under given circumstances the decrease in the turbidity value is of the first order according to the substrate. This, however, cannot be the case generally. The indispensable prerequisite is that the split products do not cause any turbidity whatsoever. Rapport, Meyer and Linker (9) have seen that the molecular weight where turbidity is no longer produced is about 8,000.

In spite of the great attention that the subject has received, the nature of the inhibition of hyaluronidase by serum is not clear. Some part of it is surely due to a competitive inhibition by related substances. Glick and Sylvén (5) have suggested that it may be due to heparin. Some tests based on this assumption are presented below. According to Glick and co-workers (4), the inhibitor is not the serum mucopolysaccharide, which contains no glucuronic acid. The possible role of glucuronides was therefore found worth of testing.

## OWN EXPERIMENTS

*Anomalies in the Turbidimetric Determination of Hyaluronidase.*

— In some preliminary tests for the experiments reported in the following paragraphs it was seen that in blank tests with the substrate and hyaluronidase the turbidity obtained after incubation was greater than with the original substrate alone. As this seemed strange, the following experiment was arranged and the degradation of the hyaluronic acid was simultaneously followed viscosimetrically in order to evaluate the possibility of enzymatic synthesis.

A substrate was prepared from human umbilical cords by extraction with physiological saline and precipitation with 2 vols. of ethanol. The product contained about 6 per cent of nitrogen and it was dissolved in physiological saline to form a solution of about 0.1 per cent. Some undissolved matter was removed by centrifugation. The solution was buffered with phosphate buffer (final conc. 0.066 *M*) to pH 6.8. (In the first experiments borate buffer was used with the same results.) Toluene was used to prevent contamination. The incubation was carried out at +37°C. with an old preparation of Treemond's testicular hyaluronidase (originally reported to contain 100 TRU/mg) in 0.1 per cent solution. The bacterial enzyme was also an old preparation from a streptococcal culture and it had been stored as a glycerine solution in the refrigerator. It was used in a dilution with physiological saline corresponding to the testicular hyaluronidase solution mentioned in the previous tests. Both enzyme solutions were used in amounts of 0.1 and 0.2 ml. The viscosimetry was carried out in Ostwald's viscosimeter. The turbidity reagent was prepared from sterile bovine serum by dilution with physiological saline in the ratio 1:50, and an equal amount of *M*-acetate buffer at pH 4.1 was added. Three ml of this reagent was added to the usual 2 ml sample of hyaluronate solution. The turbidity was measured after 5 min. in the Pulfrich photometer with filter S 61. The results are seen in Fig. 1.

*Effect of Glucuronide on Hyaluronidase.* — To evaluate the ability of glucuronide structures to act as inhibitors of hyaluronidase the effect of glucuronides was tested by the addition of phenolphthalein glucuronide, in a final concentration of 0.0001 *M*, into the incubation mixture consisting of hyaluronate solution and testicular hyaluronidase. No inhibiting effect on the enzyme was noted in the turbidimetric determination of hyaluronate. Neither did Ba-glucuronate in a concentration of 0.05 per cent exert any effect.

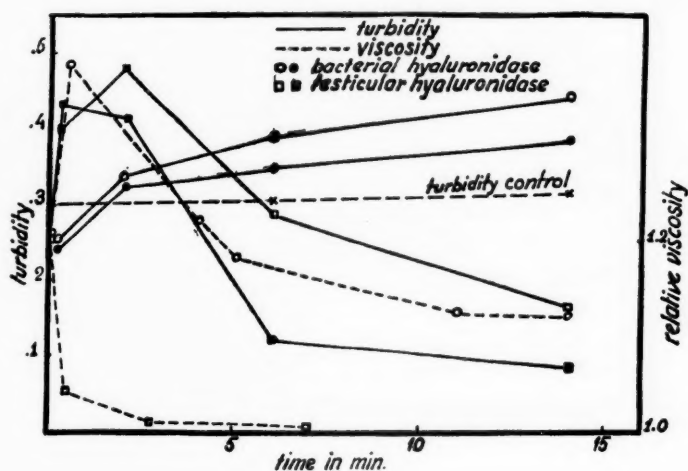


Fig. 1. — Graph showing changes in the turbidity formation and viscosity of hyaluronate solution incubated with very small amounts of old hyaluronidase preparations.

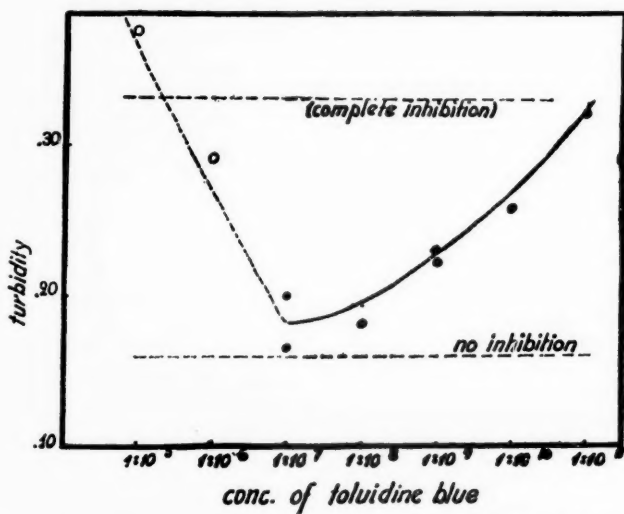


Fig. 2. — Graph showing effect of various concentrations of toluidine blue on the hyaluronidase inhibition by serum.

*Effect of Toluidine Blue on the Serum Inhibitor.* — The test was arranged as follows: Into a series of test tubes were pipetted

0.25 ml of physiological saline with various concentrations of toluidine blue and

0.25 ml of human serum.

This mixture was allowed to stand for 30 min. at room temperature and after the addition of 0.05 ml of bacterial hyaluronidase dilution left to stand a further 20 min. The enzymatic activity then remaining was tested by incubation with hyaluronate solution for 30 min. and the remaining hyaluronate determined turbidimetrically as described above. The results are seen in the Fig. 2. The values obtained with higher concentrations of toluidine blue are erroneous because of the effect of the colour of the toluidine blue itself, although a blue filter was used. In view of the probable acid structure of the inhibitor, barium chloride was also tested. Concentrations from  $10^{-2}M$  to  $10^{-8}M$  were tested but no effect on the serum inhibitor was noted. Some inhibition of the enzyme was seen with the highest concentrations.

*Tests on the Linkage of the Inhibitor with the Enzyme.* — The original enzyme solution consisted of 25 ml of the glycerine solution, which was diluted with physiological saline to 300 ml. The enzymatic activity was determined in the way described above. The turbidity value obtained indicates the remaining unchanged hyaluronic acid. The test was performed as follows:

- |   |      |
|---|------|
| 1) Incubation of 2 ml of hyaluronate solution with 0.05 ml of original enzyme solution (1) under standard conditions produced a turbidity of .....  | 0.36 |
| 2) Solution (1) mixed with half the volume of bovine serum decreased the enzyme activity, which now produced a turbidity of .....   | 0.54 |
| 3) Mixture (2) was treated with colloidal ferric hydroxide according to Rogers in order to investigate whether the serum inhibitor was adsorbed together with the enzyme, which was eluted from the precipitated ferric hydroxide with 0.2 M sodium carbonate and the eluate neutralised with hydrochloric acid. The original volume was maintained and the original hyaluronidase activity was indicated by the decrease of the turbidity to ..... | 0.30 |
| 4) Supernatant fluid after adsorption described in (3) produced a turbidity of .....  | 0.48 |
| 5) Serum alone in the same dilution as in (2) produced the same turbidity .....   | 0.53 |

A second experiment was similarly conducted, as follows:

- |   |      |
|---|------|
| 6) Original enzyme solution (1) decreased the turbidity to.....   | 0.27 |
| 7) Solution (2) was precipitated with saturated ammonium sulphate, with the addition of 10 per cent of ethanol. The precipitate was lyophilised and dissolved in the original volume. The incubation resulted in a turbidity of ..... | 0.38 |
| 8) Addition of half the volume of the serum abolished the effect, and the turbidity was .....   | 0.60 |
| 9) The addition of the original amount of enzyme to solution (7) (0.05 ml of both) did not decrease the turbidity, which remained at  | 0.38 |

#### RESULTS

Minute amounts of the enzyme increased the turbidity value at first. This anomaly was absent or much less marked if viscosimetric procedures were applied.

Toluidine blue prevents the inhibiting effect of serum on the hyaluronidase. In high concentrations it may have some inhibitory action on the hyaluronidase itself. The barium ion has no effect on the serum inhibitor.

Neither glucuronic acid nor the glucuronide seems to have any effect of physiological significance on the hyaluronidase.

Hyaluronidase is almost quantitatively adsorbed from the complex with the serum inhibitor, using colloidal iron hydroxide. When both hyaluronidase and the serum inhibitor are precipitated together and the complex lyophilised, the product is able to neutralise small amounts of the enzyme.

#### DISCUSSION

The behaviour of the turbidity is significant in practical work especially when small amounts of the enzyme are measured by turbidimetric methods. In a report which was published at the time these tests were being performed, Alburn and Whitley (1) state that when standard time is used, an increase is sometimes seen in the turbidity values. This finding gives evidence that the turbidity cannot be directly proportional to the molecular size except approximatively. The only means to avoid this anomaly is the use of a hyaluronate preparation of so low polymerisation that the split products do not contribute to the turbidity. There will then be no ascending part in the curve. This requires a mole-

cule about double the size of the product that does not produce any turbidity, or  $2 \times 8,000$ . In practical work this theoretical limit would be too low, as the first split products are in turn degraded swiftly, especially if the amount of the enzyme is not very small. If long reaction times are used the anomaly will do no harm. Although the use of a partially degraded substrate is therefore advisable, it will decrease the sensitivity of the determination of enzyme activity. When practicable, the viscosimetric method is to recommend, at least for the control.

The suggestion when the paradoxical increase in the turbidity was first observed was that there may be an enzymatic synthesis. The viscosity, however, was seen to decrease simultaneously with the increase in the turbidity. Some anomalies may be seen in the viscosity also, but these are less marked. This is due to the fact that the viscosity depends on the very large molecules relatively more than the turbidity does, which rather relates to the average molecular size. The substrates used in the turbidimetric determinations should not be native fluids.

The results in the test with toluidine blue seem to support the view presented by Glick and Sylvén (5) that the serum inhibitor may be of heparin nature. In concentrations of about  $10^{-9}$  the inhibition is prevented by the toluidine blue. Pantilitschko and Kaiser (8) have demonstrated that hyaluronidase is inhibited by polymerised chainmolecular substances with a sufficiently negative charge. Glucuronic acid or esterified sulphuric acid may supply such a charge. A polymerised state seems to be a presumption for the inhibition. If the inhibitor would be a pure carbohydrate, inhibition by serum preparations hydrolysed with pepsin or trypsin would be possible but efforts on this basis have not been successful (Kulonen, unpublished). Glucuronide alone seems to be unable to effect the inhibition of hyaluronidase, possibly because of the small molecular size or an insufficient charge.

Faber and Schmith (3) have suggested that there may exist between the hyaluronidase and its serum inhibitor a relationship where the law of mass action is valid. It seems to be supported by the loose nature of the linkage between the inhibitor and enzyme. The inhibition is reversible and cannot be due to any enzymatic action by the serum. There may exist some kind of equilibrium, where a slight addition of the enzyme is neutralised

and the system thus resembles a buffer system. It has been previously observed (6) that the amount of serum is significant to a given extent only and that increased amounts will cause only a slight effect thereafter. However, the amount required to effect equilibrium is different for various serum samples.

The nature of the complex of hyaluronidase and its inhibitor may be compared to the complex which hyaluronidase forms with ferric hydroxide at pH 5.6. The equilibrium referred to above can perhaps be compared to a buffer state where the inhibitor is to some small extent «dissociated». This «dissociating» part of the inhibitor might be replaced by hyaluronidase but the complex decomposed very easily.

#### SUMMARY

Under given conditions the turbidity values of hyaluronate solutions can be increased by incubation with hyaluronidase. The presuppositions are a small amount of the enzyme and a not too low polymerisation of the substrate. The viscosimetric determination is much less susceptible for this anomaly.

Hyaluronidase can be reversibly adsorbed with colloidal iron hydroxide from the inactive complex which is formed with the serum inhibitor. The enzyme and the serum inhibitor form a state of equilibrium, where small additions of either hyaluronidase or serum are inactivated. The action of the inhibitor seems to be prevented by toluidine blue. Glucuronide has no effect on hyaluronidase.

#### REFERENCES

1. ALBURN, H. E., and WHITLEY, R. W.: *J. Biol. Chem.* 1951:192:379.
  2. DORFMAN, A.: *J. Biol. Chem.* 1948:172:377.
  3. FABER, V., and SCHMITH, K.: *Scand. J. Clin. Lab. Investigation* 1950:2:298.
  4. GLICK, D., GOOD, T. A., KELLEY, V. C., WINZLER, R. J. and MEHL, J. W.: *Proc. Soc. Exp. Biol. Med.* 1949:71:412.
  5. GLICK, D., and SYLVÉN, B.: *Science* 1951:113:388.
  6. KULONEN, E.: *Acta Physiol. Scand.* 1951:24:suppl. 88.
  7. KULONEN, E.: Unpublished experiments.
  8. PANTILITSCHKO, M., and KAISER, E.: *Biochem. Z.* 1951:322:137.
  9. RAPPORT, M., MEYER, K., and LINKER, A.: *J. Biol. Chem.* 1950:186:615.
  10. ROGERS, H. J.: *Biochem. J.* 1946:40:583.
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## THE NORADRENALINE AND ADRENALINE CONTENT OF HUMAN FETAL ADRENAL GLANDS AND AORTIC BODIES

by

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(Received for publication May 14, 1952)

In the embryonic development of the adrenals the chromaffin cellular tissue begins to form during the seventh week, and after the fourth month we can already speak of an adrenal medulla. After this time immigration of chromaffin tissue continues during the whole fetal period. The chromaffin tissue occurs abundantly also in the aortic bodies (paraganglia in the region of the abdominal aorta = Zuckerkandls body) which at a certain stage of fetal development forms an important part of this system (15). Lelkes (18) has shown, by biological methods, that as early as towards the end of the third fetal month the human adrenal gland contains adrenaline, and beginning with the fifth month the substance can also be demonstrated in Zuckerkandls body.

The adrenaline content of fetal adrenal glands and aortic bodies has been studied in experimental animals by biological and chemical methods to a relatively insignificant extent (9, 10) and corresponding studies on man are scarce (3, 17, 18). New prospects of the fetal chromaffin system have been opened up by studies on the adrenal glands, performed on experimental animals (1, 2, 5, 6, 7) and human adults (22), showing that noradrenaline, in addition to adrenaline, is the second neurohormone produced by the adrenal medulla. The noradrenaline content of the human fetal adrenals has hardly been studied at all. Since, now, the biological reactions

of infant and management of delivery have awakened new interest, we have attempted to make a chemical determination of the noradrenaline and adrenaline in the human fetal adrenals and Zuckerkandl's body at different stages of the intrauterine period. In some cases attempts were made to correlate the content and the condition of the fetus at the moment of birth.

#### MATERIAL AND METHODS

Our series, collected in 1950—51 at the Women's Clinic, was studied at the Department of Medical Chemistry of the University of Helsinki. The series comprised a total of ten aborted fetuses (therapeutical or spontaneous abortion, weight below 600 g), five immatures (600—1249 g) and eight prematures (1250—2499 g), as well as five fully developed fetuses, i.e. 28 cases in all. Only those fetuses were included whose time of death and cause of death were known. None of the fetuses had been dead for longer than one hour *in utero*.

The aborted fetuses were autopsied immediately after birth, the others within 12 hours of it. Up to then the fetuses were kept in a refrigerator. At autopsy the adrenal glands were carefully brought forward. Both glands were handled separately. Since in several cases the aortic bodies could not be clearly distinguished, the gelatinous superficial coats of the abdominal aorta were prepared from the site of beginning of the inferior mesenteric artery in the caudal direction up to the branch of the common iliac arteries. The specimens were stored in a refrigerator ( $-1^{\circ}\text{C}$ ).

Chemical quantitative determinations of adrenaline and noradrenaline could only be performed on part of the material. This was due to the circumstance that noradrenaline and adrenaline of the adrenal glands and aortic bodies under examination could only be performed by the chemical micromethod. In 19 cases the micromethod was used for chemical studies.

Noradrenaline and adrenaline of the fetal adrenals and aortic bodies were determined in a trichloroacetic extract from which the trichloroacetic acid was separated by elution with ether, according to v. Euler's and Hamberg's iodine oxidation reaction (6), performing oxidation both at a pH of 4 and a pH of 6. The pH's were increased by a sodium acetate-acetic acid buffer solution.

Readings of the red noradrenochrome and adrenochrome colour which appeared were performed in a microcuvette 5 cm long with a Stufen photometer (filter S 53). The blank was carried out under similar conditions, without iodine oxidation. Since the readings were low, great care was taken to have both the extracts and the solutions employed absolutely transparent in the micro-determination.

## RESULTS

The results are illustrated in tables 1—5. In order to present a coherent picture, we divided our series into two groups, the first comprising fetuses weighing over 800 g and the second those under 800 g. The groups correspond approximately to the last or third trimester and the middle or second trimester of pregnancy, respectively.

TABLE 1

TOTAL AMOUNT OF NORADRENALINE AND ADRENALINE IN BOTH ADRENALS

Case Number	Weight of Fetus, g	Length of Fetus, cm	Total Noradrenaline, $\mu$ g	Total Adrenaline $\mu$ g	Total Catechols $\mu$ g	Noradrenaline/Total Catechols, Per Cent
1	4270	50	41	62	103	40
2	4170	51	129	0	129	100
3	3700	50	104	0	104	100
4	2370	49	5	86	91	6
5	2035	46	37	25	62	60
6	2020	44	6	0	6	100
7	1910	43	41	0	41	100
8	1800	44	69	14	83	83
9	1315	39	31	39	70	44
10	840	35	80	0	80	100
11	830	35	48	12	60	80
12	800	35	19	0	19	100
13	630	30	15	10	25	60
14	600	32	45	21	66	71
15	580	31	0	0	0	—
16	570	30	12	10	22	56
17	420	25	0	0	0	—
18	320	26	23	18	41	56
19	230	20	37	4	41	90

TABLE 2

CONCENTRATION OF NORADRENALINE AND ADRENALINE IN ADRENALS

Case Number	Weight of Fetus, g	Weight of Adrenals (both), g	Noradrenaline mg per cent	Adrenaline mg per cent
1	4270	7.7	A 0.6 B 0.4	0.7 1.7
2	4170	11.2	A 1.5 B 0.9	0 0
3	3700	5.5	A 1.7 B 2.5	0 0
4	2370	7.2	A 0.1 B 0	0.9 1.4
5	2035	4.9	A 0.8 B 0.7	0.6 0.4
6	2020	3.9	A 0.4 B 0	0 0
7	1910	2.9	A — B 1.4	— 0
8	1800	3.8	A 1.9 B 1.7	0 0.7
9	1315	5.3	A 0.5 B 0.7	0.4 1.4
10	840	2.6	A 2.6 B 3.5	0 0
11	830	2.8	A 3.0 B 1.3	0.7 0.4
12	800	2.7	A — B 1.2	— 0
13	630	2.4	A 0.2 B 1.0	0.4 0.4
14	600	1.1	A 5.9 B 2.8	1.9 1.9
15	580	2.8	A — B 0	— 0
16	570	1.6	A 0 B 1.7	0 1.4
17	420	1.5	A — B 0	— 0
18	320	1.1	A 1.8 B 1.7	1.4 2.2
19	230	0.7	A 2.7 B 8.4	1.0 0

## ADRENAL GLANDS

*Amounts of Noradrenaline and Adrenaline (table 1).* — The total amounts of noradrenaline and adrenaline (total catechols) in the adrenal glands were from 0 to 129  $\mu$ g. In spite of the restricted series one can observe an increase of the absolute quantities of total catechols during the intrauterine period, although not always in correlation with the increasing weight of the fetus. In the last trimester of pregnancy the total catechols had an average of 71  $\mu$ g, with a corresponding average of 27  $\mu$ g in the second trimester.

When comparing the distribution of noradrenaline and adrenaline in our series, one finds that in several cases the amount of noradrenaline in the fetal adrenals considerably surpasses that of adrenaline. The average amount of noradrenaline per fetus was 51  $\mu$ g in the last period of gestation and 19  $\mu$ g in the second, the corresponding figures for adrenaline being 20  $\mu$ g and 9  $\mu$ g.

*Noradrenaline and Adrenaline Contents (table 2).* — The average content of noradrenaline during the second and third trimester was 2.2 and 1.2 mg per 100 ml and of adrenaline 0.9 and 0.4 mg per 100 ml. The adrenaline content surpassed 2 mg per 100 ml only in one case, whereas the noradrenaline content was over 2 mg per 100 ml in four cases.

## AORTIC BODIES

*Amounts of Noradrenaline and Adrenaline (table 3).* — The average quantity of total catechols in the second trimester was 11  $\mu$ g, whereas in the last trimester it was 40  $\mu$ g. Consequently, the increase in catechol substances of the aortic bodies can be regarded as approximately corresponding to the gain in weight of the fetus.

The preponderance of noradrenaline in regard to adrenaline is even more marked in the aortic bodies than in the adrenals. During the last trimester of fetal life the aortic bodies had an average of 34  $\mu$ g noradrenaline and 6  $\mu$ g adrenaline, the corresponding figures for the second trimester being 8  $\mu$ g and 3  $\mu$ g.

*Noradrenaline and Adrenaline Contents.* — As already mentioned, it proved to be necessary, in order to include the whole

TABLE 3

TOTAL AMOUNT OF NORADRENALINE AND ADRENALINE IN AORTIC BODIES

Case Number	Weight of Fetus, g	Total Noradrenaline, $\mu$ g	Total Adrenaline, $\mu$ g	Total Catechols, $\mu$ g	Noradrenaline/Total Catechols, Per Cent
1	4270	68	10	78	87
2	4170	65	0	65	100
3	3700	10	0	10	100
4	2370	14	7	21	67
5	2035	25	20	45	56
6	2020	46	0	46	100
7	1910	100	27	127	79
8	1800	53	0	53	100
9	1315	10	5	15	67
10	800	10	0	10	100
11	830	0	6	6	0
12	800	8	0	8	100
13	630	5	5	10	50
14	600	3	8	11	27
15	580	30	0	30	100
16	570	5	2	7	71
17	420	6	0	6	100
18	320	0	6	6	0
19	230	6	0	6	100

aortic bodies, to prepare the surrounding tissues. In three fourths of the cases the noradrenaline content surpassed 4 mg per 100 ml, whereas the adrenaline content exceeded this border value only in one fourth of the cases. As a rule, the contents were higher than those of the adrenal glands.

## ADRENAL GLANDS AND AORTIC BODIES

The absolute quantity of total catechols in both adrenals glands and aortic bodies (table 4) increases up to the end of the fetal period, having an average of 39  $\mu$ g in the second and 111  $\mu$ g in the third trimester.

Since the fetuses showed considerable difference in weight, we also calculated the total catechols of adrenal glands and aortic bodies per kilogram of body weight. This value seemed to decrease before the end of the fetal period. In the second trimester it was

TABLE 4

TOTAL AMOUNT OF CATECHOLS (= NORADRENALINE AND ADRENALINE) IN ADRENALS AND AORTIC BODIES

Case Number	Weight of Fetus, g	Total Catechols, $\mu\text{g}$	Total Catechols in Aortic Bodies/Total Catechols in Adrenals and Aortic Bodies. Per Cent	Total Catechols $\mu\text{g/kg}$ of Body-weight
1	4270	181	43	42
2	4170	194	34	47
3	3700	114	9	31
4	2350	112	19	48
5	2035	107	42	53
6	2020	52	88	26
7	1910	168	76	88
8	1800	136	39	76
9	1315	85	18	65
10	840	90	11	107
11	830	66	9	78
12	800	27	30	34
13	630	35	29	56
14	600	77	14	128
15	580	30	100	52
16	570	29	24	51
17	420	6	100	14
18	320	47	13	146
19	230	47	13	204
Adult	ca. 60000	ca. 8000	—	ca. 130

TABLE 5

Case Number	Weight of Fetus, g	Total Catechols in Adrenal and Aortic Bodies $\mu\text{g}$	Relative Asphyxia before Death of Fetus
1	4270	181	+
2	4170	194	+++
3	3700	114	+++
4	2350	112	++
5	2035	107	++++
6	2020	52	++
7	1910	168	+++
8	1800	136	+
9	1315	85	++

95  $\mu\text{g}$  catechols per kg of body weight, whereas the figure was 58  $\mu\text{g}$  for the last trimester.

In comparing the total catechols of the aortic bodies to the joint amount of total catechols in both adrenals and aortic bodies, in the second and last trimester of pregnancy the total catechols of the aortic bodies amount to approximately two fifths of the total catechols in both organs.

#### TOTAL CATECHOLS OF THE FETUS IN DIFFERENT DELIVERIES

Fetal asphyxia has been estimated as +, ++ and +++, according to the course of delivery. The series comprised only 9 cases which provided a basis for assessing the total catechols in the fetal organs under discussion and the course of delivery. These cases did not show any correlation between duration and difficulties of labour on the one hand, and total catechols in these organs on the other. For instance, the first case was a precipitate delivery, whereas labour was prolonged in the second. Both mothers were multiparae. In both cases the amount of total catechol substances was almost identical. Similarly cases 6 and 7 (elderly primiparae), in both of which cesarean section was performed. Case 7, in which operation was preceded by 38 hours of inadequate pains (indication: fetal asphyxia) had three times the total catechols of case 6, in which operation was performed after 10 hours of pains.

#### COMMENT

1. The fetal adrenals in the main part of our series contained relatively abundant amounts of noradrenaline, which exceeded half of the catechol substances in the adrenals. On the other hand, e.g., the fetus of the cow also has relatively high quantities of noradrenaline in the adrenals (22). This is in contradiction with the condition in the adult human adrenal as in cow, in which the amount of noradrenaline is on an average only 20 per cent of the total catechols (22). Noradrenaline is also found in considerable amounts in medullary adrenal tumours (9, 13). One can therefore assume that the growing embryonic tissue which can be taken to include the developing adrenal medulla of the fetus just as well as adrenal medullary tumours, only contains noradrenaline which,

under the circumstances, is evidently unable to be methylated into adrenaline. It can also be assumed that during fetal life the adrenal medulla has not yet specialised in acting as a reserve of adrenaline, instead of which it can be functionally compared to the peripheral sympathetic ganglia. Possibly a store of the adrenaline hormone is superfluous in the intrauterine period.

In comparison to the adult, the adrenal catechol content is very low in fetal life, only  $1/30$ — $1/40$  as compared to the normal human adrenals, in which an adrenaline content of 72 mg per 100 ml and a noradrenaline content of 14 mg per 100 ml have been found in sudden deaths (22, 26), but, on the other hand, fetal aortic bodies have a considerably high catechol content, which generally exceeds that of the sympathetic ganglia in the adult. The adrenaline concentration in the fetal adrenal was found to have an average of 0.4—0.9 mg per 100 mg, with the figures 1.2—2.2 mg per 100 ml for noradrenaline. In fact, these glands have a considerable size in fetal life (4), due to the characteristic fetal cortex. In our series the weight of the adrenals in large fetuses corresponds nearly to the weight of the adult adrenal glands (26). For instance, in the fourth month of pregnancy, a period during which first determinations were made, the ratio in the weight of the adrenal bodies and the kidneys was 1: 1, at the time of birth still 1: 3, whereas the same ratio in the adult is 1: 28. Since it has been determined the catechol contents of the whole adrenal gland, i.e. the cortex also, and not only the adrenal medulla, it is obvious that the total catechols of such a large adrenal will be relatively low. Although the catechol content of the adrenals is small, however, the amount of catechol substances calculated per kg of body weight is moderate.

2. The second characteristic feature of the fetal chromaffin system is constituted by the aortic bodies, which contain abundant noradrenaline, exceeding in several cases that of the adrenals. Indeed, these bodies can be compared to the sympathetic ganglia, which mainly contain noradrenaline alone, irrespective of the period. In addition to catechols of the fetal adrenals, the aortic bodies contain a considerable part of the catechol substances, on an average two fifths of the total catechols in the adrenals and aortic bodies of the fetus. Therefore, the aortic bodies, beside the adrenal glands, play a fairly significant role as a source of catechol

substances, and a much more significant role than that belonging to ordinary sympathetic ganglia. Moreover, the catechol concentration in aortic bodies is much higher as a rule than in the sympathetic ganglia or fetal adrenals. The question why the fetus develops such a relatively large store of catechol substances outside the adrenals can only be studied against the evolutionary background already referred to.

3. In the larger fetuses the total catechol substances of the adrenal glands and aortic bodies per kg of body weight did not quite attain, as a rule, the figures found in smaller fetuses. In general, however, the total catechols of these organs increase towards the end of the fetal period. The amount may be affected in individual cases by numerous physiological factors, in regard to which no conclusions can be drawn on the basis of a small series (such as birth mechanism and the reaction ability of the chromaffin system).

4. What is the role of chromaffin tissue in fetal life, taking into consideration the relatively high total catechol amount, especially noradrenaline per weight unit, is a question which has not yet been answered. The task of noradrenaline in the organism is to regulate continually the tonus of the blood vessels, although it does not really participate in the metabolic functions, as does adrenaline (15, 16). Instead, adrenaline is a substance which is secreted in emergency reactions, when abruptly increased functioning is necessary to the organism, and when adrenaline regulates the blood supply to the organs in function and mobilises the chemical energy reserves (12, 17). Since the mother supplies the fetus with oxygen and other nutrient substances, it is conceivable that in emergency reactions adrenaline does not have the same significance for the fetus as for the adult. The fetus does not exercise any greater activity in the womb and is fully dependent on the mother during the process of delivery. On the other hand, noradrenaline, which is a substance abundantly secreted during fetal life, may have its own significance as a principle regulating the vascular (23) tonus but having little effect on metabolism (11). In any case, the fetus is so rich in catechols that if need arises they are sufficient to maintain the tonus of the fetal circulation.

Our study, which only embraces a small number of cases, does not show whether or not the fetal chromaffin system is capable

of continuous formation and secretion of catechol substances, primarily noradrenaline, for the benefit of the organism. In spite of prolonged asphyxia, we found in some cases abundant amounts of catechol substances in the aortic bodies and adrenal glands. It must be said that in the cases studied by us there was no marked correlation between difficulties of labour and catechol concentrations. Whereas in the adult the noradrenaline and adrenaline concentration in the adrenal glands show a very marked decrease, e.g. in association with prolonged shock (8, 12).

The extent to which the fetal chromaffin system participates in emergency reactions is still unknown. We know for instance that all endocrine mechanisms are not fully developed during fetal life (20, 21). It is true that the fetal adrenal cortex also reacts to ACTH by secreting cortical hormones for the protection of the cells of the organism, but since the pituitary does not respond to adrenaline, it seems that the impulses emerging from the fetal chromaffin tissue are incapable of regulating, through the pituitary, the activity of the adrenal cortex, a phenomenon to which a central position has been ascribed in the adaptation mechanism of the organism. E.g., the impulses of the sympathetic nervous system or an injection of adrenaline do not produce elaboration of ACTH by the pituitary, as seen in the fullterm newborn (16, 19).

#### SUMMARY

The writers have studied the occurrence of noradrenaline and adrenaline in adrenal glands and aortic bodies during different periods of fetal life. The series comprised 28 cases with a weight ranging from 12 up to 4270 g. Chemical determinations were performed by the micromethod in 19 cases. In collecting the series particular consideration was given to its freshness. The following observations were made:

1. During the last trimester of the intrauterine period the total catechols of the adrenals averaged 71  $\mu\text{g}$  and in the second trimester 27  $\mu\text{g}$ . The corresponding figures for the aortic bodies were 40  $\mu\text{g}$  and 11  $\mu\text{g}$  respectively. The ratio between the catechols of aortic bodies and of adrenals remained constant throughout fetal life. The total catechols of the adrenals and aortic bodies per

kilogram of body weight were somewhat less than the corresponding amount in normal adult adrenals (in cases of sudden death).

2. A considerable part of total catechols in the adrenals consisted of noradrenaline; in three fourths of the cases more than one-half was noradrenaline, i.e. notably more than in adult humans. The preponderance of noradrenaline in the aortic bodies was even more pronounced, amounting to an average to  $\frac{5}{6}$  of the total catechols during the last trimester.

3. The catechol concentration of the fetal adrenal however, was found to be considerably less than of the adult gland (1:50), which is partly due to the large adrenal cortex, characteristic of fetal life.

4. No clear correlation could be established between catechol concentrations and difficulties of labour. Normal catechol amounts were found even in prolonged asphyxia.

#### REFERENCES

1. BERGSTRÖM, S., v. EULER, U. S., and HAMBERG, U.: *Acta Physiol. Scand.* 1950:20:101.
2. BÜLBRING, E., and BURN, J. H.: *Brit. J. Pharm. and Chemother.* 1949:4:202.
3. DANISCH, F.: *Verhandl. Deutsch. Path. Ges.* 1926:21:222.
4. EKHOLM, E., and NIEMINEVA, K.: *Acta Paediatrica* 1950:39:67.
5. v. EULER, U. S.: *Erg. Physiol.* 1950:46:261.
6. v. EULER, U. S., and HAMBERG, U.: *Acta Physiol. Scand.* 1949:19:74, 207.
7. v. EULER, U. S., and HAMBERG, U.: *Nature* 1949:163:642.
8. GOLDENBERG, M., FABER, M., ALSTON, E. J., and CHARGAFF, E. C.: *Science* 1949:109:534.
9. GOLDZIEHER, M. A.: *The Adrenal Glands*, F. A. Davis Company, Publishers, 1946.
10. HARTMAN, F. A., and BROWNELL, K. A.: *The Adrenal Gland*, London, Henry Kimpton, 1949.
11. HELVE, O., and PEKKARINEN, A.: *Ann. Med. Exper. et Biol. Fenn.* 1952:30.
12. HOLTZ, P.: *Die Pharmazie* 1950:5:49.
13. HOLTON, P.: *Nature* 1949:163:217.
14. HOLTZ, P.: *Ztschr. f. ges. inn. Med.* 1951:6:65.
15. IVANOW, Z.: *Ztschr. f. Anat. u. Entwicklungsgeschichte* 1927:84:238.
16. JAILER, J. W., WONG, A. S. H., and ENGLE, E. T.: *J. Clin. Endocrinol.* 1951:11:186.
17. KRAMER, D.: *Monatschr. f. Kinderheilk.* 1918:14:531.
18. LELKES, Z.: *Endokrinologie* 1941:23:259.

19. MOORE, C.: *J. Clin. Endocrinol.* 1950:10:942.
  20. NIEMINEVA, K.: *Acta Paediatrica* 1950:39:366.
  21. NIEMINEVA, K.: *Ann. Med. Exper. et Biol. Fenn.* 1950:28:262.
  22. PEKKARINEN, A.: 1952 (to be published).
  23. PEKKARINEN, A., and ARO, L.: *Ann. Chir. et Gynecol.* 1952:41:69.
  24. PEKKARINEN, A., and HORTLING, H.: *Acta Endocrinol.* 1951:6:193.
  25. READ, C. H., VENNING, E. H., and RIPSTEIN, M. P.: *J. Clin. Endocrinol.* 1951:10:845.
  26. UOTILA, U., and PEKKARINEN, A.: *Acta Endocrinol.* 1951:6:23.
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## CITRIC ACID METABOLISM IN ACUTE ANOXIA IN DOGS

by

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(Received for publication May 17, 1952)

In a short communication in 1949 Lewis (6) showed that in acute anoxia in dogs the amount of citric acid in plasma rose for a short time even more than a hundred times. The citric acid binds the calcium and prevents the blood from clotting. The amount of calcium did not, however, change much. In order to investigate this phenomenon we have made experiments on dogs. We have, besides citric acid, estimated the contents of glucose, pyruvic acid, and lactic acid in blood. In stead of using arterial blood, as Lewis (6) did, we have investigated venous blood.

### PROCEDURES AND METHODS

The dogs for this experiment were taken from three different litters a, b and c, with 5, 9, and 5 puppies. The dogs in the a and c litters were of Finnish mixed breed, the b litter being of alsatian-rottweiler mixed breed. The dogs were 3—4 months old.

The investigation was performed as follows:

The dogs got ether anesthesia or were anesthetized i.v. with nembutal. As soon as possible the deep jugular vein was prepared out. A cannula was put in position for withdrawing the samples. A rubber mask was drawn over the dog's head and nitrogen was lead directly from a bomb to the mask. The first effect was an elaboration of the breathing, but death from lack of oxygen followed within a few minutes. The blood samples were taken immediately at the beginning of the anaesthesia and then as often as possible

after the nitrogen had been led into the mask. When the heart contraction had ceased, some samples were drawn directly from the heart.

Citric acid was analyzed according to the method of Pucher (8). Lactic acid was estimated according to Barker and Summerson (1), pyruvic acid according to Friedeman and Haugen (2), and glucose according to Somogyi (9).

#### RESULTS

The results of the experiments are given in diagrams. Besides the above results we found that oxygen in blood decreased extremely fast when nitrogen was given, practically to nil, the values being 1–0.1 volume per cent. This shows that we have been able to procure real anaerobic conditions in our experiments.

The citric acid proved changeable. In the first group (Fig. 1 a) it increased regularly, the highest value being 4 times higher than the value we started with. The increase of the values commenced very soon after the nitrogen was given. The highest value was attained some time after the breathing had ceased, and after this a sudden fall followed. In the second group (Fig. 1 b), the alsatian-rottweiler mixed breed litter, the citric acid did not increase. The third litter, again Finnish mixed breed, showed clearly an increasing citric acid value in one case (Fig. 1 c). Some of these dogs, 16, 17, and 19, got, differently from the dogs in group a and b, nembutal anaesthesia.

Simultaneously made pyruvic acid estimations showed regularly decreasing values (Fig. 2 a, b and c). Neither the type of anaesthesia nor the breed seemed to influence the values. The amount of pyruvic acid started decreasing immediately after the nitrogen had been given. The small variations in the diagrams depend apparently on errors in the methods.

We also determined the lactic acid for the 2nd and 3rd litters. Contrary to the pyruvic acid the lactic acid in the blood increased regularly immediately after the nitrogen had been given (Fig. 3 b and c). The same thing happened with the glucose in the blood (Fig. 4 b and c) with a few exceptions. The increase of the glucose in the blood began, however, only when the dogs had breathed nitrogen for some minutes, when the circulation already showed signs of ceasing.

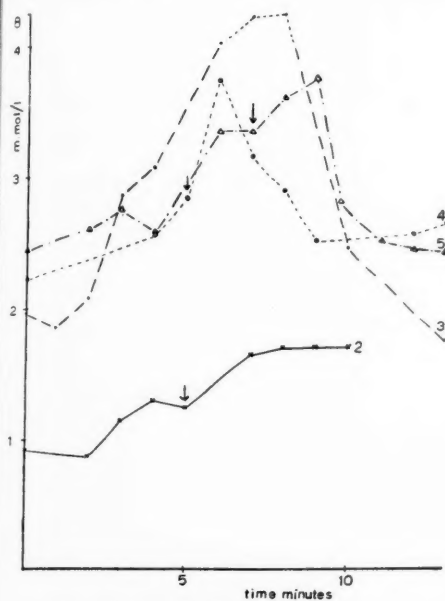


Fig. 1 a. — Citric acid, m.mol/l blood. Dogs 2, 3, 4, and 5. ↓ Cessation of breathing.

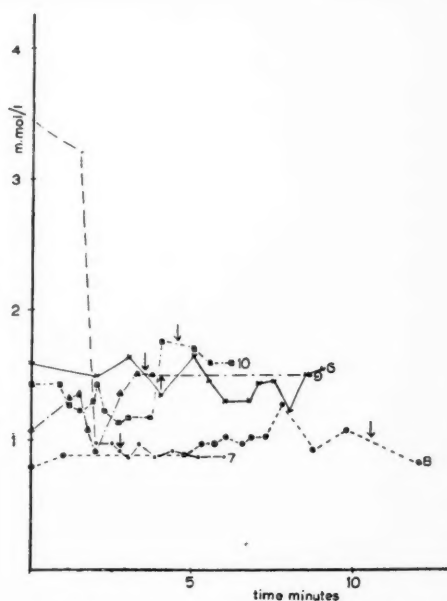


Fig. 1 b. — Citric acid m.mol/l blood. Dogs 6, 7, 8, 9, and 10.

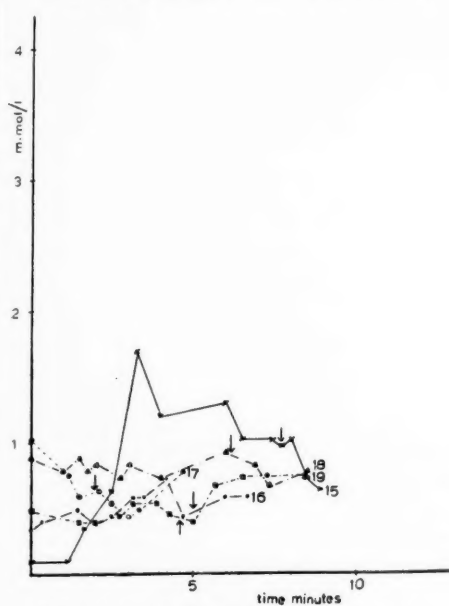


Fig 1 c. — Citric acid, m.mol/l blood. Dogs 15, 16, 17, 18, and 19.

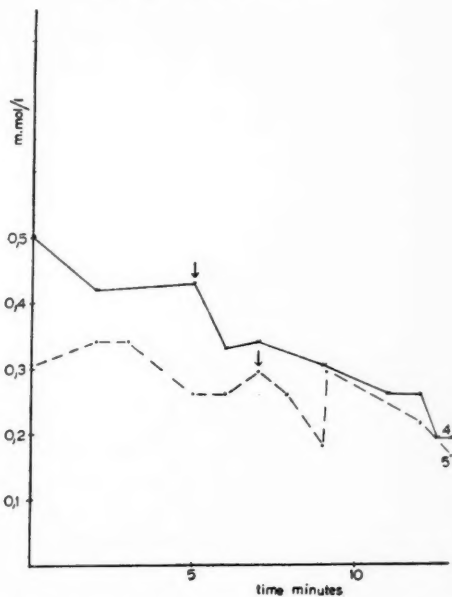


Fig. 2 a. — Pyruvic acid, m.mol/l blood. Dogs 4 and 5.

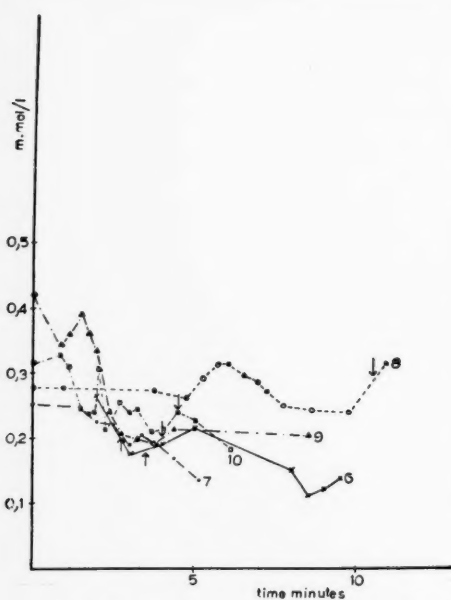


Fig. 2 b. — Pyruvic acid, m.mol/l blood.  
Dogs 6, 7, 8, 9, and 10.

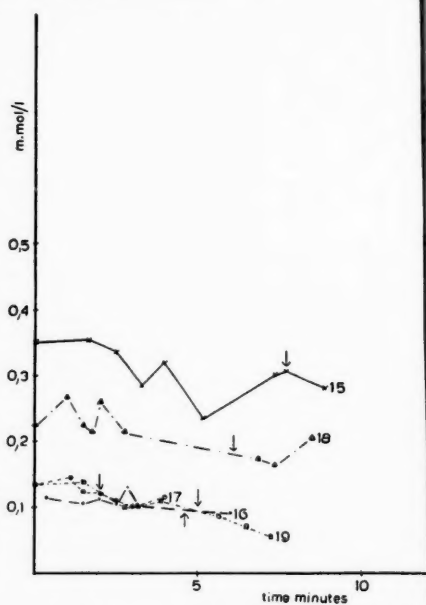


Fig 2 c. — Pyruvic acid, m.mol/l blood.  
Dogs 15, 16, 17, 18, and 19.

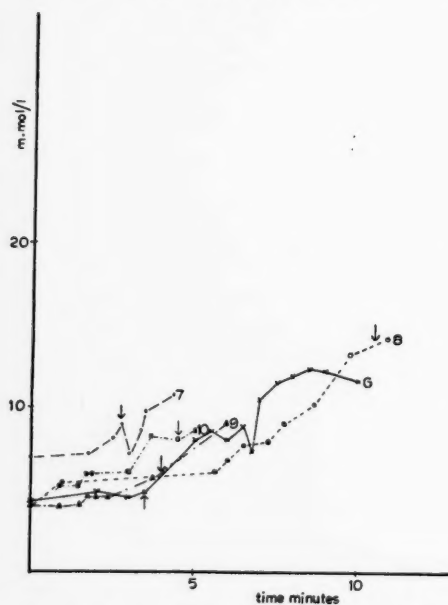


Fig. 3 b. — Lactic acid, m.mol/l blood.  
Dogs 6, 7, 8, 9, and 10.

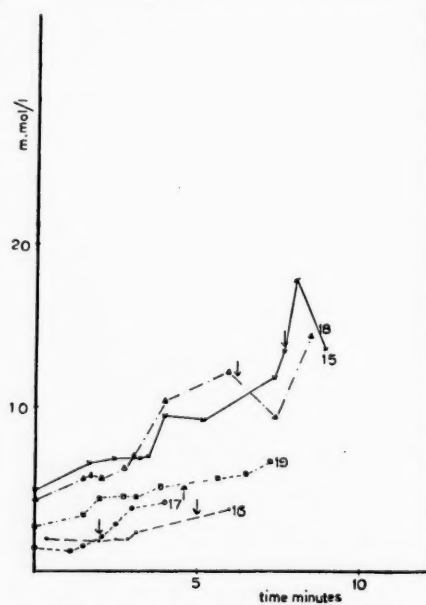


Fig. 3 c. — Lactic acid, m.mol/l blood.  
Dogs 15, 16, 17, 18, and 19.

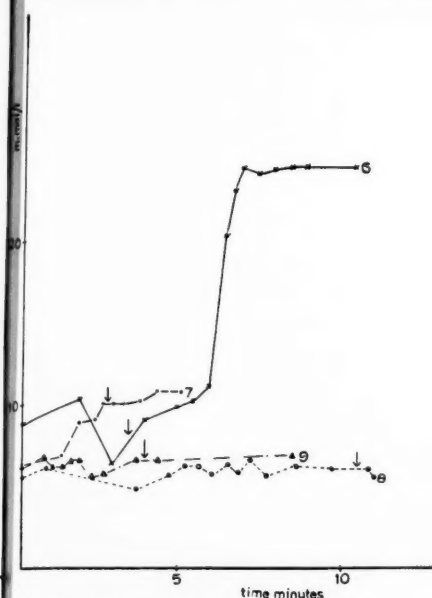


Fig. 4 b. — Glucose, m.mol/l blood.  
Dogs 6, 7, 8, and 9.

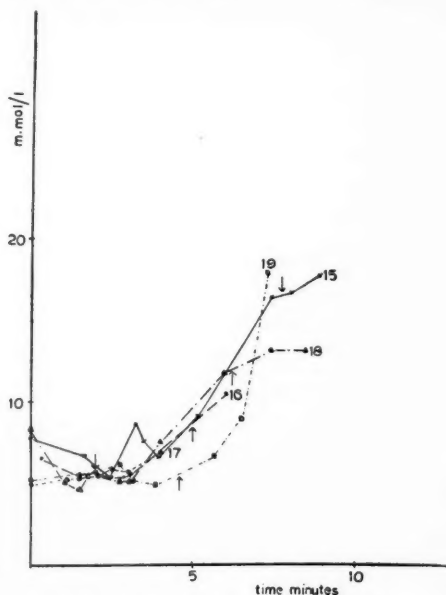


Fig. 4 c. — Glucose, m.mol/l blood.  
Dogs 15, 16, 17, 18, and 19.

#### DISCUSSION OF THE RESULTS

It is known that in the last phases of the anoxia, when breathing difficulties are beginning to appear in the organism and in the blood, an acidotic state develops (5), which may change into a deeper acidosis than in any other condition. This originates partly from the concentration of lactic acid, but, according to many investigators, the concentration of lactic acid alone is not sufficient to explain the deepness of the acidotic state.

It is also known that if the oxygen pressure decreases, pyruvic acid concentrates in the blood. The increase of lactic acid is, however, more pronounced, the proportion lactic acid-pyruvic acid increases. (4). In deep acute anoxia the pyruvic acid appears to decrease in the blood of dogs, as our investigations show. Apparently lactic acid is formed from pyruvic acid, the lactic acid thus acting as a hydrogen acceptor.

It is difficult to explain the concentrating of citric acid in the venous blood in dogs in some of our investigations, as also Lewis (6)

has shown in corresponding experiments, and it is equally difficult to explain the rather sudden decrease. It is a well-known fact that citric acid is formed from pyruvic acid and oxalacetic acid probably during a condensation process. It is shown in experiments with tissues (3) that citric acid is formed from  $\alpha$ -ketoglutaric acid also under anaerobic conditions. In accordance with Ochoa's (7) investigations the formation of citric acid can occur, under certain circumstances, quite contrary to the ordinary circulation of the cycle, directly from the  $\alpha$ -ketoglutaric acid. It may be supposed that the concentrating of the citric acid in the blood in our experiments occurred according to Ochoa's reaction. Lewis (6) has shown that the aconitic acid in the blood does not increase simultaneously. In this connection there is no reason to discuss the more exact position of the citric acid in the cycle, about which there are different opinions.

#### SUMMARY

Acute anoxia in dogs, produced experimentally by giving them nitrogen gas, is investigated in 15 cases. The content of citric acid, lactic acid, pyruvic acid, and glucose in the blood is followed. The results are discussed.

#### REFERENCES

1. BARKER, S. B., and SUMMERSON, W. H.: *J. Biol. Chem.* 1941:138:535.
  2. FRIEDEMANN, T. E., and HAUGEN, G. E.: *J. Biol. Chem.* 1943:147:415.
  3. HALLMAN, N.: *Acta Physiol. Scand.* 1940:2:suppl. IV.
  4. HAVEL, R. J., and WATKINS, E.: *Circulation* 1950:2:536.
  5. KOEHLER, A. E., BRUNQUIST, E. H., and LOEVENHART, A. S.: *J. Biol. Chem.* 1925:64:313.
  6. LEWIS, J. H.: *J. Clin. Invest.* 1949:28:796.
  7. OCHOA, S.: *J. Biol. Chem.* 1945:159:243.
  8. PUCHER, G. W.: *J. Biol. Chem.* 1944:153:133.
  9. SOMOGYI, M.: *J. Biol. Chem.* 1945:160:69.
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## EFFECT OF DESOXYCORTICOSTERONE ACETATE ON RED CELL POTASSIUM AND SODIUM IN INFANTS

by

NIILO HALLMAN and HILKKA TÄHKÄ

(Received for publication May 17, 1952)

It is known that desoxycorticosterone acetate (DCA) produces retention of sodium and chloride in the organism, with a simultaneous loss of potassium. Reverse changes occur in adrenal insufficiency and also after adrenalectomy. The electrolytic changes have been investigated both by means of balance tests and tissue analysis. On the other hand, no studies of red cells, which are always easily obtainable and whose changes can be continually observed, have been performed on man. Hegnauer and Robinson (6) as well as Nilson (8) have found in cats and dogs that red cell potassium increases and sodium decreases subsequent to adrenalectomy, i.e. changes which also occur in other tissues.

We present below the results obtained with red cell potassium and sodium in connection with the administration of DCA to infants. Some of the cases were simultaneously treated by extra potassium.

### MATERIAL AND METHOD

The series comprised 10 infants with ages ranging from 5 weeks to 11 months. While the studies were in progress the babies were kept on a diet accordance with their age, five were given extra potassium in addition at some stage of the treatment.

The DCA preparation used was DOCA (Organon) or PERCORTEN (Ciba). The potassium and sodium analyses were performed

TABLE 1  
POTASSIUM AND SODIUM CONTENT OF PLASMA AND ERYTHROCYTES DURING DCA TREATMENT

Number Age Birthweight Diagnosis	Date	Weight	Erythrocytes		Plasma		Dosage of DCA	Remarks
			K mEq/l	Na mEq/l	K mEq/l	Na mEq/l		
Case 1. 6 months 2500 g Microcephalus	29.3.	6020	98	17	5.4	—	29.3.—4.4.	Ordinary food. Condition good during the treatment. Discharged condition unchanged.
	31.3.	6120	100	17	5.0	140	2 × 5 mg daily	
	3.4.	6220	94	17	5.0	141	5.4.—14.4.	
	5.4.	6280	100	—	5.3	—	2 × 10 mg daily	
	8.4.	6480	92	17	5.5	148		
	11.4.	6450	88	17	5.5	139		
	13.4.	6490	94	17	5.0	132		
	15.4.	6550	102	15	4.5	146		
Case 2. 11 months 4000 g Dystrophica post gastro enteritidem	21.4.	6550	—	—	—	—		Ordinary food. Condition unchanged during the treatment. Regular gain in weight started 6 weeks after finishing treatment.
	14.4.	6900	—	—	—	—	20.4.—23.4.	
	19.4.	6880	98	17	4.8	150	2 × 5 mg daily	
	22.4.	6870	85	19	4.3	—	24.4.—27.4.	
	24.4.	6860	82	15	5.2	141	2 × 10 mg daily	
	26.4.	6870	95	18	4.4	145		
	28.4.	6970	88	18	4.5	143		
	2.5.	6800	90	23	4.4	140		
Case 3. 4 weeks 3200 g Monstrum	27.5.	6650	103	20	4.9	149		Ordinary food. Condition good during the treatment. Discharged condition unchanged.
	4.8.	7800	—	—	—	—	20.4.—23.4.	
	20.4.	3560	97	16	—	—	2 × 5 mg daily	
	22.4.	3600	108	15	4.3	—	24.4.—28.4.	
	24.4.	3650	86	17	4.9	139	2 × 10 mg daily	
	26.4.	3610	92	16	—	—		
	28.4.	3600	96	19	4.4	135		
	30.4.	3510	—	—	—	—		
	10.5.	3950	—	—	—	—		

Case 4. 7 months 3110 g Fibrosis pancreatis	1.4. 24.4. 26.4. 28.4. 2.5. 5.5. 17.5.	4000 4220 4380 4350 4500 4340 4370	— 99 89 98 96 97	— 18 16 14 16 17	— 5.0 4.3 5.2 4.7 4.4	— 142 135 137 140	24.4.—2.5. 2 × 5 mg daily	Ordinary food. Condition unchanged during the treatment. Discharged condition unchanged.
Case 5. 3 months 3440 g Dystrophia post gastroenteritidem.	30.10. 3.11. 6.11. 7.11. 9.11. 11.11. 13.11. 15.11. 20.11. 26.11.	3900 4030 4110 4170 4220 4330 4320 4400 4470 4520	97 95 97 98 97 91 91 99 99	16 17 21 16 14 15 15 16 17	4.4 4.9 5.5 5.0 4.8 4.9 5.0 5.0 4.7	128 130 134 140 141 131 137 133 135	3.11.—6.11. 1 × 5 mg daily 7.11.—10.11. 2 × 5 mg daily 11.11.—14.11. 2 × 10 mg daily	Ordinary food. Condition good during the treatment. Discharged as convalescent.
Case 6. 3 months 4000 g Rachitis.	2.2. 20.2. 24.2. 28.2. 5.3. 8.3. 10.3. 13.3. 20.3.	5720 6180 6230 6390 6330 6530 6470 6600 7180	— 99 96 93 100 99	— 20 19 20 18 18	— 6.4 5.4 4.9 5.5 5.4	— 142 133 136 135	20.2.—23.2. 1 × 5 mg daily 24.2.—27.2. 2 × 5 mg daily 28.2.—4.3. no treatment 5.3.—8.3. 2 × 5 mg daily	2.2.—4.3. ordinary food. 5.3.—8.3. Ordinary food and »Darrow-Solution»* 150 ccm/day. Discharged as convalescent.

\* »Darrow-Solution» contains: NaCl 2.6 g, KCl 3.7 g, Glucose 20.0 g in Aqua dest. ad 1000.0.

(Table 1 cont.)

Number Age Birthweight Diagnosis	Date	Weight	Erythrocytes		Plasma		Dosage of DCA	Remarks
			K mEq/l	Na mEq/l	K mEq/l	Na mEq/l		
Case 7. 1 1/2 months 3450 g Convalescentia post gastritidem.	15.2.	3350	—	—	—	—	20.2.—23.2.	15.2.—4.3. ordinary food.
	20.2.	3520	100	20	5.5	149	1 × 5 mg daily	5.3.—8.3. ordinary food
	24.2.	3700	101	21	5.6	155	24.2.—27.2.	and »Darrow-Solution» 100
	28.2.	3880	94	18	4.7	150	2 × 5 mg daily	ccm/day. Discharged as
	5.3.	4000	100	18	4.7	138	28.2.—4.3.	convalescent.
	9.3.	4160	103	21	5.3	145	no treatment	
	11.3.	4070	—	—	—	—	5.3.—8.3.	
	13.3.	4170	103	17	5.0	155	2 × 5 mg daily	
Case 8. 8 months 2000 g Idiotia.	15.3.	4220	—	—	—	—		
	10.2.	7700	—	—	—	—	24.2.—27.2.	10.2.—8.3. ordinary food.
	24.2.	7850	100	16	5.4	165	1 × 5 mg daily	9.3.—13.3. ordinary food
	28.2.	8230	95	15	4.8	139	28.2.—4.3.	and »Darrow-Solution» 100
	5.3.	8300	98	15	6.5	128	2 × 5 mg daily	ccm/day. Discharged con-
	9.3.	8230	100	16	5.8	138	5.3.—8.3.	dition unchanged.
	13.3.	8430	103	16	6.0	143	no treatment	
	15.3.	8310	—	—	—	—	9.3.—13.3.	
	17.3.	8330	99	17	6.0	138	2 × 5 mg daily	
	27.4.	9500	—	—	—	—		

Case 9. 6 months 3330 g Atrophica post gastroenteritidem.	25.5.	3260	95	24	3.6	130	25.5.—2.6. 1 × 5 mg daily	Ordinary food and «Dar- row-Solution» 100 ccm/day 25.5.—7.6. Condition un- changed during the treat- ment. Regular gain in weight started about 1 month after finishing the treatment.
	27.5.	3270	98	23	3.8	—	3.6.—7.6.	
	31.5.	3440	101	16	4.7	138	2 × 5 mg daily	
	3.6.	3400	106	16	4.4	134		
	7.6.	3600	104	19	4.0	127		
	10.6.	3420	—	—	—	—		
	14.6.	3500	98	17	3.8	130		
	20.6.	3500	98	18	4.8	141		
	8.8.	4050	—	—	—	—		
Case 10. 3 ½ months 3550 g Fibrosis pancreatis.	1.6.	3470	—	—	—	—	7.6.—15.6. 1 × 5 mg daily	Ordinary food and «Dar- row-Solution» 100 ccm/day 7.6.—20.6. Condition un- changed during the treat- ment. Death.
	7.6.	3400	96	17	4.2	138	16.6.—20.6.	
	10.6.	3420	93	16	4.0	136	2 × 5 mg daily	
	12.6.	3450	96	18	4.5	127		
	14.6.	3550	98	16	4.7	130		
	16.6.	3540	97	23	4.4	144		
	20.6.	3480	104	23	4.3	150		
	22.6.	3350	—	—	—	—		
	24.6.	3400	96	19	4.4	140		
	9.8.	2580	—	—	—	—		

by a flame photometer (4). When studying the red blood cells, heparin free from sodium was added to blood taken under anaerobic conditions, and centrifugation (2000 revolutions/min.) was performed as soon as possible after the withdrawal, first for 30, then for 10 minutes, now and then removing plasma by means of a pipette. The red cells were then pipetted by a calibrated pipette from the bottom of the centrifuge tube, and hemolysis was performed in a graduated bottle. No determinations of the water content of red blood cells were performed. The figures show sodium and potassium per volume of red blood cells.

### RESULTS

The results are shown on Table 1. We see that there was a regular fall of red cell potassium on a normal diet (Cases 1—8), and the fall was generally more pronounced under a larger dose of DCA. If administration was continued over a longer period, the values usually returned to normal. The lowest level of red cell potassium was 81 mEq/l, the normal values, when this method is applied, surpassing 98 mEq/l (5).

The red cell sodium values generally remained unchanged. Nor could any constant differences be obtained in regard to plasma potassium and sodium.

In those cases (cases 6—10) which, in addition to ordinary food, were treated by extra potassium, there was no decrease in red cell potassium. Nor did the plasma potassium reveal any simultaneous changes worthy of note.

It seems that the nutritional state of the infant as such plays no part in the results. As illustrated by the appended table, 4 of the babies in our series (cases 1, 3, 6, 8) had a more or less normal weight, three were slightly underweight (cases 2, 5, and 7), and three were strongly underweight (cases 4, 9, 10). The weight rose invariably during administration of DCA. After the suspension of the medication a slight loss of weight occurred more often than not. The weight of normal infants developed thereafter in the usual way. The gain in weight of atrophic babies after DCA was discontinued was equally poor as before. In other words, DCA failed to bring about a normal continuous gain in weight in these babies.

## DISCUSSION

As shown by the results obtained, administration of DCA produced in both normally developed and underweight infants a fall in red cell potassium, provided these babies were on a diet of ordinary food consistent with their ages. The results is in agreement with the change known to be brought about by DCA in the cells of the organism in general. In spite of the circumstance that the weight of all the infants increased during the DCA medication, which indicates retention of water and sodium, there was no rise of the red cell sodium. Nor was there any significant elevation of plasma sodium and potassium.

As already mentioned, Hegnauer and Robinson (6), as well as Nilson (8) have found an increase of red cell potassium and a decrease of sodium in the cat and the dog after adrenalectomy, which is the reverse of DCA medication. Yet we cannot compare these animals with man, if only for the reason that under normal conditions, both animals have a low red cell potassium and high sodium level, while the reverse is the case with man. The time of administration of DCA in our experiments varied between 8 and 17 days, a period which we feel to be sufficiently long to produce any possible changes.

Earlier investigators (3, 7) advocate the administration of DCA to atrophic babies in order to accelerate their gain in weight. According to others, abundant amounts of potassium should be given simultaneously to counteract the loss (1, 2). According to our observations, the administration of DCA to atrophic infants only results in a temporary gain in weight. If extra potassium is given simultaneously, the fall of red cell potassium, otherwise pronounced, does not occur. It is possible that extra potassium administered with DCA prevents the occurrence of potassium deficiency in the organism in general, produced as a rule by the said hormone. Consideration should be given to the circumstance that even in those cases in which potassium was not given simultaneously with DCA, the red cell potassium, after an initial fall, gradually returned to normal. This suggests the function of a regulatory mechanism in the organism itself, which consequently occurs in atrophic babies as well.

## SUMMARY

A study is made of the effect of desoxycorticosterone acetate on red cell potassium and sodium in 10 infants. The dosage varied from 5 to 20 mg daily and the period of administration from 8 to 17 days. Part of the babies had a normal weight, part were underweight.

On ordinary diet there was an invariable fall of red cell potassium, which, however, frequently returned to normal if the experiment was continued. If extra potassium was given simultaneously with ordinary food, the said change could not be produced. No significant alterations could be observed either in cell sodium or plasma potassium and sodium.

Under the administration of desoxycorticosterone acetate the weight increased in all cases. Likewise, suspension of the medication was followed by a decrease in weight. In atrophic infants the gain in weight never progressed any better after the administration of the hormone than before it.

## REFERENCES

1. BARTA, L.: *Ann. Paediatr.* 1948:171:158.
  2. BIGLER, J. A., and TRISMAN, H. S.: *Am. J. Dis. Childr.* 1951:82:548.
  3. FORSSELL, P.: *Ann. Med. Int. Fenn.* 1947:36:247.
  4. HALLMAN, N., and LEPPÄNEN, V.: *Suomen Kemistilehti (Finn.)* 1949: B 22:55.
  5. HALLMAN, N., and LEPPÄNEN, V.: *Ann. Med. Int. Fenn.* 1952:41:21.
  6. HEGENAUER, A. H., and ROBINSON, E. J.: *J. Biol. Chem.* 1936:116:769.
  7. HENI, F.: *Z. Exp. Med.* 1941:108:427.
  8. NILSON, H. W.: *Am. J. Physiol.* 1937:118:620.
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## ANOMALOUS TOXIN PRODUCED BY *CORYNEBACTERIUM* *DIPHTHERIAE*

by

LAURI JÄNNES<sup>1</sup>

(Received for publication June 19, 1952)

In experiments made for enhancement of diphtheria toxin yields on a casein hydrolysate medium, toxins with some extraordinary properties were obtained. Since these toxins reacted quite normally with diphtheria antitoxin in the flocculation test, corresponding toxoids being, however, almost devoid of immunizing potency, the publication of the results seemed justified.

The medium used in these experiments was the routine substrate prepared for diphtheria toxin production in this laboratory, i.e. a weak casein hydrolysate medium (3) with added cystine and essential nutrients [nicotinic acid, pimelic acid and  $\beta$ -alanine (4)] The NaCl content of this medium is 0.5 per cent. The nitrogen content is adjusted to 0.11 per cent with glycine and glutamic acid. A previously tested amount of yeast autolysate is used as the iron and activator source (11). In the yeast autolysate, obviously, not only the optimum quantity of iron, but also some additional necessary factors are introduced into the medium. A more regular high toxin production (usually 120—150 L<sub>t</sub> doses/ml) is thus obtained with several P.W.8. strains of *C. diphtheriae*. The principal effective agent in yeast autolysate seems — besides iron — with all probability to be thiamine (1).

The toxin production of a certain Toronto variant (*C. diphtheriae* P.W.8./M.D.6./0—J2, in our collection) appeared to be un-

<sup>1</sup> Aided by a grant from the Sigrid Juselius Foundation.

usually tolerant to the increasing iron content of the substrate. The amount of added yeast autolysate, and thus the content of iron in the medium, could be raised from the normal optimum about thirtyfold while apparent toxin yields up to 300  $L_t$  doses/ml were obtained. The fact that supra-optimal iron concentration stimulates the production of bacterial protein while toxin production is depressed is already known (5, 8). In this case, however, the protein produced behaved in the flocculation test consistently, exactly as normal diphtheria toxin does, and only the agar gel diffusion technique (6) revealed the difference: several diffuse precipitation lines were formed instead of the one clearcut line typical for good toxins<sup>1</sup>. A batch of these anomalous toxins was converted to toxoid and this subsequently tested for immunizing properties. The antitoxin titre in the blood of guinea pigs immunized with this toxoid remained on a level which was about one tenth of the level obtained with normal toxoids.

Some characteristics of a good normal diphtheria toxin and of an anomalous toxin produced simultaneously on same batch of medium, differing only in yeast autolysate content and, consequently, in iron content, are given below (Table 1).

TABLE 1

Toxin	Yeast Autolysate <sup>2</sup> ml/150 ml Medium		$L_t$ /ml	$K_t(L_t^{50})$	Protein-N mg/ml	Dlm ml
	1 % Solution	6 % Solution				
Normal ..	1.4	—	120	7	0.097	0.001
Anomalous	—	7.0	230	13	0.325	0.2

The electrophoretic patterns of these two toxins are represented in Figs. 1 and 2.

The electrophoretic runs were made in phosphate buffer (ionic strength 0.1, pH 7.10) at + 2° C from total proteins precipitated from the toxins with  $(NH_4)_2SO_4$ . The protein concentration was 2 per cent and the potential gradient during the run 4.5 volt/cm.

<sup>1</sup> As antitoxin both the international flocculation standard and a sub-standard supplied to us by courtesy of Wellcome Research Laboratories, Beckenham, England, reacted similarly.

<sup>2</sup> The yeast autolysate solutions were made from spray dried yeast autolysate supplied by A/S Synthetic, Grindsted, Denmark.

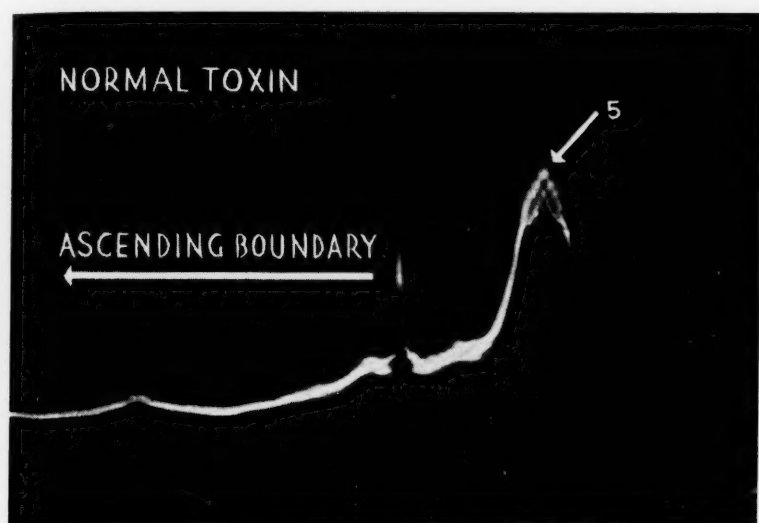


Fig. 1.

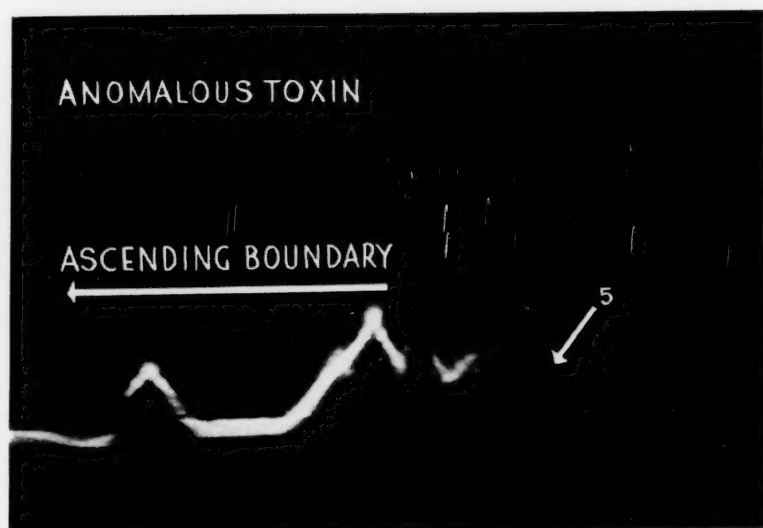


Fig. 2.

As anticipated on account of the difference in protein nitrogen (Table 1), almost all components of the normal toxin are evidently enhanced in the anomalous one, except the component which is represented by the peak marked 5 in both pictures. This presumably represents the pure toxin moiety of the protein complex produced by the diphtheria bacterium (7, 9) and had — in these runs — respectively the mobilities  $3.8 \times 10^{-5}$  cm<sup>2</sup>/volt sec (normal toxin) and  $4.0 \times 10^{-5}$  cm<sup>2</sup>/volt sec (anomalous toxin). The calculated percentage of this component was 49.3 per cent for the normal toxin and 8.8 per cent for the anomalous toxin.

It seems evident that the addition of bigger amounts of yeast autolysate to the medium enhances — with this strain — the production of other proteins so closely related to real diphtheria toxin that they interfere with the flocculation reaction between toxin and antitoxin, and false  $L_t$  values are obtained<sup>1</sup>. It appears thus somewhat dangerous to force the toxin production of *C. diphtheriae* too high, especially if the  $L_t$  titres estimated in the flocculation test are used as the sole criterium for the value of the toxin.

I am indebted to Mr A. Louhivuori, M. A., State Serum Institute, Helsinki for carrying out the electrophoresis of the toxins in the Tiselius apparatus and for the preparation of the photographs.

#### REFERENCES

1. DREW, RUTH, and MUELLER, J. H.: J. Bact. 1951:62:549.
2. EISLER, M.: Schweiz. Z. für allg. Path. und Bakteriöl. 1950:XIII,6:708.
3. HOLT, L. B.: Brit. J. Exp. Path. 1948:29:335.
4. MUELLER, J. H., and MILLER, P. A.: J. Immunol. 1941:40:21.
5. NORLIN, G.: Acta Path. et Microbiol. Scand. 1943:Suppl. L.
6. OUCHTERLONY, Ö.: Arkiv Kemi, Mineral. Geol. 1948:26 B:1.
7. PAPPENHEIMER, A. M., JR., LUNDGREN, H. P., and WILLIAMS, J. W.: J. Exp. Med. 1940:71:247.
8. PAPPENHEIMER, A. M., JR.: J. Biol. Chem. 1947:167:251.
9. PETERMAN, M. L., and PAPPENHEIMER, A. M., JR.: J. Phys. Chem. 1941:45:1.
10. POPE, C. G., STEVENS, M. F., CASPARY, E. A., and FENTON, E. L.: Brit. J. Exp. Path. 1951:32:246.
11. SIEVERS, O., and JÄNNES, L.: Acta Path. Scand. 1945:XXII,2:201.

<sup>1</sup> The coexistence of several protein components in ordinary and even in purified diphtheria toxins has already been pointed out (2, 10).

## EIN EINFACHER UND GENAUER FLAMMENPHOTOMETER ZUR BESTIMMUNG DER BLUTALKALIMETALLE

von

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(Eingegangen am 20 Juni 1952)

In den letzten Jahren hat die Flammenphotometrie in immer zunehmendem Umfange Anklang in klinischen Laboratorien gefunden. Die schnelle und exakte Serienbestimmung der Blutalkalimetalle, besonders des Natriums und Kaliums, ist eine Forderung, die an jedes gut ausgerüstetes modernes Krankenhauslaboratorium gestellt wird. Die Untersuchung der Kalium- und Natriumkonzentration im Blutplasma und den roten Blutkörperchen ist nicht nur von Bedeutung bei der Aufrechterhaltung der Flüssigkeitsbilanz vor und nach chirurgischen Eingriffen und bei inneren Krankheiten, die zu Störungen derselben führen können, sondern auch zur Erkennung von Dysfunktionen der endokrinen Organe und vorallem zur Überwachung der ACTH- bzw. Cortisonbehandlung. Ferner sind die Alkalimetallbestimmungen im Blut auch in der Kinderheilkunde, besonders bei der Behandlung schwerer Gastroenteritiden, von Aus-schlag gebender Bedeutung, wie u.a. Hallman und Leppänen (1) hervorgehoben haben.

Die modernen fabriksmässig hergestellten Flammenphotometer sind mit mehreren Nachteilen behaftet, die ihren allgemeinen Gebrauch in klinischen Laboratorien erschweren oder sogar unmöglich machen. Der grösste Teil der Apparate erfordert, um eine ausreichend heisse Flamme zu erzielen, Azetylen- oder Propangas, welches in der

<sup>1</sup> Unterstützt durch ein Stipendium der Buchdruckerei »Tampereen Kirjapaino Oy (Aamulehti)».

Klinik schon wegen der Feuer- und Explosionsgefahr unpraktisch ist. Ferner ist die Konstruktion der grösseren und besseren Flammenphotometer so kompliziert, dass ihre Inbetriebnahme und Überwachung nicht vom gewöhnlichen Laboratoriumpersonal oder von Ärzten vorgenommen werden kann. Auch ist ein grosser Teil der im Handel befindlichen Flammenphotometer für ein viel breiteres Anwendungsgebiet konstruiert worden und eignet sich daher oft nicht wegen zu geringer Empfindlichkeit für Mikroanalysen im Blut. Schliesslich ist auch zu sagen, dass die besseren, grossen Flammenphotometer einen sehr hohen Anschaffungspreis haben.

Die grosse Unsicherheit, welche zur Zeit noch in klinischen Laboratoriumskreisen herrscht über die geeignetste Apparatur kam u.a. deutlich zum Ausdruck auf dem nordischen Kongress für klinische Chemie und klinische Physiologie in Aulanko 1950, wo man sich nicht für ein spezielles Modell entscheiden konnte und wo man sich auch prinzipiell nicht einig wurde über die Frage, ob ein Flammenphotometer mit einer Photozelle genügt oder ob es besser ist eine Apparatur mit zwei Photozellen zu benutzen, von denen die eine als Kompensationszelle dient und die andere die eigentliche Messung ausführt (3).

Im Anschluss an den erwähnten Kongress wurde im Herbst 1950 für das Laboratorium des Kivelä Krankenhauses der Flammenphotometer von der Firma Dr. B. Lange, Berlin, Modell 2 mit Kompressor und Multiflex-Galvanometer angeschafft (Apparat von Riehm-Lange (4)). Dieser Apparat erschien vorteilhaft nicht nur wegen dem niedrigen Anschaffungspreise, sondern auch wegen der Einführung der ziemlich selektiven Interferenzfilter anstatt des komplizierten optischen Prismasystemes. Leider erwies sich aber dieser Apparat nach ausgedehnten Versuchsserien als nicht genügend empfindlich für Mikroanalysen im Blut. Es wurde daher in enger Zusammenarbeit der Verfasser, von denen T. Mettinen Schwachstromexpert ist, ein vollkommen neuer Flammenphotometer gebaut unter Anwendung mehrerer Teile des Apparates von Dr. B. Lange. Der Bau des Flammenphotometers ist im Prinzip kurz folgender: Mittels konstantem Luft- und Gasdruck wird eine stabile Leuchtgasflamme erzehlt, deren Emission durch zwei Interferenzfilter monochromatisiert und von zwei Vacuum-Photozellen registriert wird. Die erhaltenen elektrischen Impulse werden gegeneinander kompensiert und die entstandene Differenz wird durch

einen Messverstärker geleitet und mit dem Multiflex-Galvanometer gemessen.

Im Folgenden soll die von den Verff. angewendete Apparatur in Detail beschrieben werden:

1. *Der Luftkompressor.* — Als Kompressor wurde die von Dr. B. Lange angegebene Medvak-Pumpe (Arthur Pfeiffer, [16] Wetzlar) angewendet, welche mit einem Wechselstrommotor von 80 W arbeitet. Diese Drehkolbenpumpe fördert 1000 Liter Druckluft pro Stunde mit sehr gleichmässigen Druck und kann bis 0.6 atü dauernd belastet werden. Eine eigene kleine Druckpumpe eignet sich besser für den Flammenphotometer als ein stählerner Druckluftbehälter. Der Kompressor wird auf einem getrennten Tisch angebracht um keine mechanischen Störungen im Galvanometer-Ausschlag hervorzurufen.

2. *Die Manometer.* — Als Druckluft- und Leuchtgasmanometer wurde die von Dr. B. Lange gelieferte Einrichtung benutzt. Beide Manometer sind auf dieselbe Grundplatte montiert. Die U-Röhre des Druckluftmanometers ist in 0.9 atü eingeteilt mit Unterteilungen von 0.1 atü und wird mit Quecksilber gefüllt. Die kleinere U-Röhre für den Leuchtgasdruck ist in mm Wasserdruck eingeteilt und wird mit Wasser gefüllt. Neben beiden U-Röhren sind verschiebbare Marken angebracht, die auf den erfordernten Druck eingestellt werden um kleine Druckänderungen sofort sichtbar zu machen.

3. *Die Regulationsventile.* — Die Ventile für sowohl Druckluft als auch Leuchtgas sind ebenfalls die von Dr. B. Lange gelieferten. Das Regulationsventil der Druckluft besteht aus einem aufschraubbaren Topf, der mit Watte gefüllt wird zum Filtrieren der Luft, und einem Präzisionsventil mit verstellbarer Metallspitze. Das Leuchtgasventil besteht wiederum aus einer Staudüse mit zwei Anschlüssen für den Manometer zur Kontrolle des Gasdrucks.

4. *Der Vernebler.* — Als Vernebler wird ebenfalls der von Dr. B. Lange gelieferte Ultra-Zerstäuber nach Lederle benutzt, dessen genauer Bau aus der Abb. 1 hervorgeht. Der Vernebler wird mit einem Stativ an derselben Grundplatte befestigt wie der Flammenphotometer (Abb. 2). Die zu analysierende Probe wird von einer nach unten gebogenen Kapillare aufgesaugt in die Verneblungskammer, wo die neben die Kapillare einmündende Druckluft die Flüssigkeit fein vernebelt. Der untere Teil des Verneblers ist zu einer Kugel abgerundet, von der eine Glasröhre zum Brenner führt. An

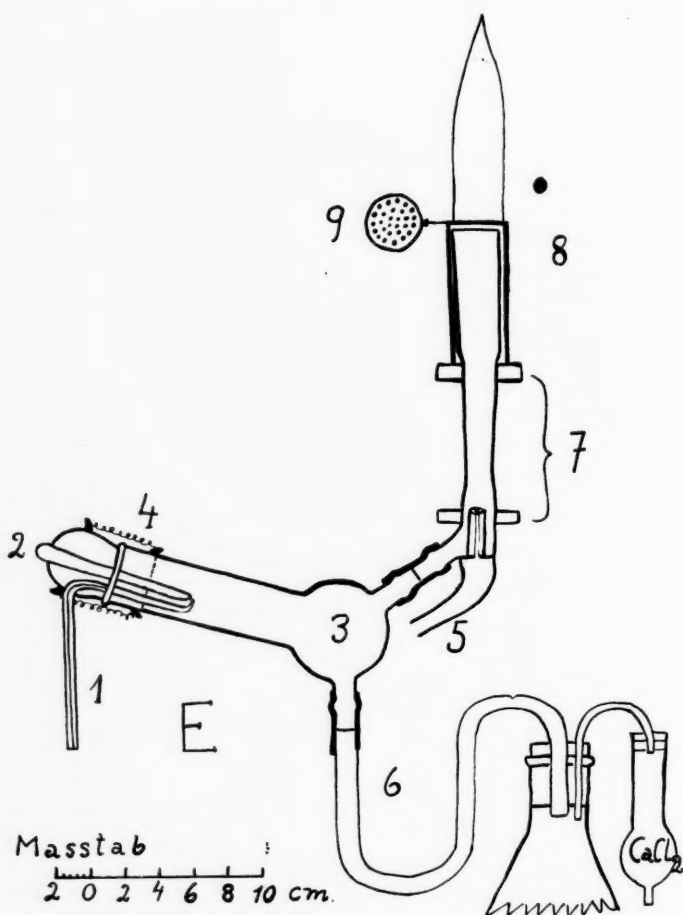


Abb. 1. — Der Vernebler und Brenner<sup>1</sup> (vgl. Abb. 2 Teil E).

die Kugel ist ausserdem eine U-Röhre angeschlossen zur Entfernung der überflüssigen Lösung und zur Regulierung des Druckes in der Verneblungskammer. Der Vernebler verbraucht ca 10 ml Flüssigkeit pro Minute. Die durch die U-Röhre abfliessende Flüssigkeit wird in einen Erlenmeyerkolben aufgenommen, der mit einer Chlorcalcium-Röhre verschlossen ist zur Verhinderung der Verdunstung von Feuchtigkeit.

5. Der Brenner. — Der Brenner in dem Apparate von Dr. B.

<sup>1</sup> Erklärungen gegeben in Abb. 2.

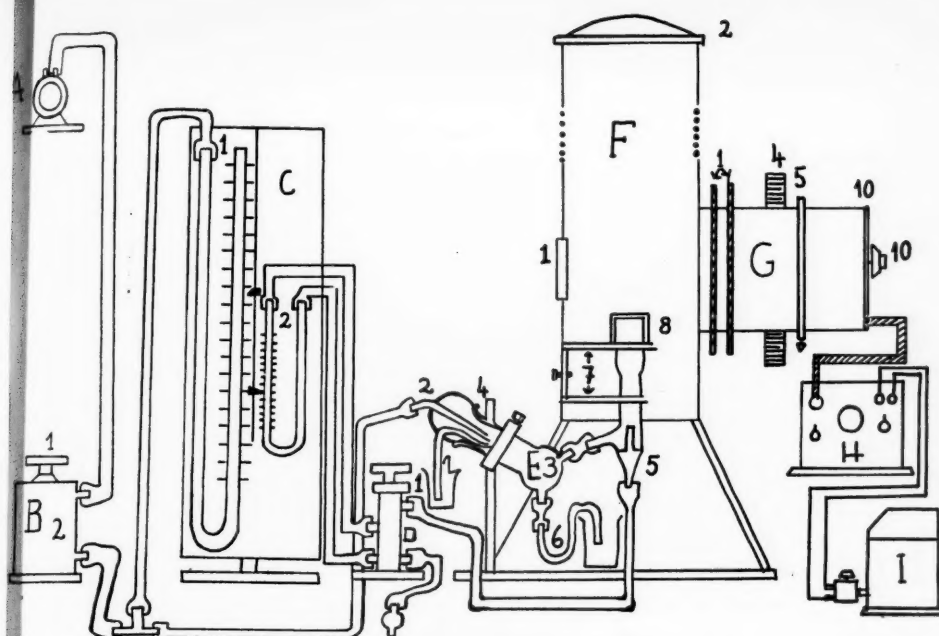


Abb. 2. — Schematisierte Gesamtansicht des Flammenphotometers.

*Erklärung der Teile:*

A. Kompressor

B. Druckluftfilter und Ventil

1. Nadelventil

2. Filtrationstopf gefüllt mit Watte

C. Manometerbrett

1. Druckluftmanometer (Hg)

2. Leuchtgasmanometer ( $H_2O$ )

D. Leuchtgasventil

E. Vernebler und Brenner

1. Kapillare zur Aufsaugung der Analyse

2. Drucklufttröhre

3. Vernebelungskammer

4. Schliff

5. Leuchtgasröhre

6. U-röhre (Druckregulierung und Wasserableitung)

7. Befestigungsklammern

8. Metallhülse des Brenners (Messing)

9. Durchlöchernde Endplatte der Metallhülse (Kupfer)

F. Brennergehäuse

1. Türöffnung zum Brenner

2. Deckelaufsatz des Brennergehäuses

G. Nebenrohr des Flammenphotometers

1. Kühlplatten

2. Kondensorlinse

3. Fensteröffnungen

4. Filterdrehscheibe

5. Blendscheibe mit keilförmigen Schlitz

6. Lithiumfilter

7. Vacuumphotozellen

8. Elektronenröhren

9. Silicagelbehälter

10. Verschlussplatte mit Dunkelstromeinstellung

H. Verstärker

I. Multiflex-Galvanometer mit Vorschaltkasten

Lange gab eine zu kleine Flamme mit einem zu grossen blauen Innenkegel. Der Bau des von uns konstruierten Brenners geht aus der Abb. 1 hervor; er entspricht einem gewöhnlichen Meker-Brenner. Die Gasröhre ist aus Glas und auf sie lose aufgesetzt befindet sich der eigentliche Brenner, ein Messingmantel mit angeschweisster feindurchlöcherter Kupferplatte. Die Glasröhre hat einen Durchmesser von 28 mm, der Metallansatz entsprechend einen Durchmesser von 29—30 mm. Dieser Brenner gibt mit Leuchtgas und einem Gasdruck von 40 mm Wasser und Luftdruck von 0.6 atü eine ca 10 cm hohe, breite Flamme mit nur ca 2 mm grossen blauen Innenkegeln. Der heisseste und stabilste Teil der Flamme befindet sich gleich über den blauen Innenkegeln. Der Brenner wird so in den Flammenphotometer montiert, dass dieser Teil der Flamme benutzt wird.

6. *Der eigentliche Flammenphotometer.* — Das Photometerrohr des ursprünglichen Apparates von Dr. B. Lange besteht aus dem Verbrennergehäuse und einem Nebenrohr mit dem optischen System. In unserem Apparate wurde das Verbrennergehäuse beibehalten, mit der Abänderung, dass unter den Deckelaufsatz des Stahlrohres ein dünner 50 cm langer Flammenschutz aus Blech eingefügt wurde. Diese Verlängerung des Stahlrohres erwies sich erforderlich um die Hitzeüberleitung auf das Nebenrohr mit dem optischen System so weit als möglich zu vermeiden. Die Nebenröhre, welche das optische und einen Teil des elektrischen Systems enthält, wurde völlig neukonstruiert. Der äussere Bau geht aus der Abb. 2 hervor. Die Länge der Nebenröhre aus Stahl gleicher Dicke wie das Brennergehäuse beträgt 215 mm, der Durchmesser 100 mm. Zur Steigerung der Wärmeverdunstung wurden gleich am Anfang der Röhre zwei Stahlscheiben von ca 190 mm Durchmesser angeschweisst. Der Abstand zwischen diesen beiden Kühlscheiben beträgt 28 mm. Distal von den Scheiben befindet sich auf der linken Seite der Nebenröhre ein Ansatz für die Filter-Drehscheibe (Abb. 2 und 3), und etwas weiter vorne auf der rechten Seite ein kleinerer Ansatz für die Blendscheibe der Kompensationszelle. Auch diese beiden Ansätze vermehren natürlich die Kühlfläche. Das Nebenrohr ist mit einer Pakelitscheibe verschlossen, an deren inneren Seite ein Teil des optischen und elektrischen Systems angebracht ist. — Das ganze Photometerrohr ist wie in dem Apparat von Dr. B. Lange auf einer stabilen Grundplatte neben dem Vernebler angebracht (Abb. 2).

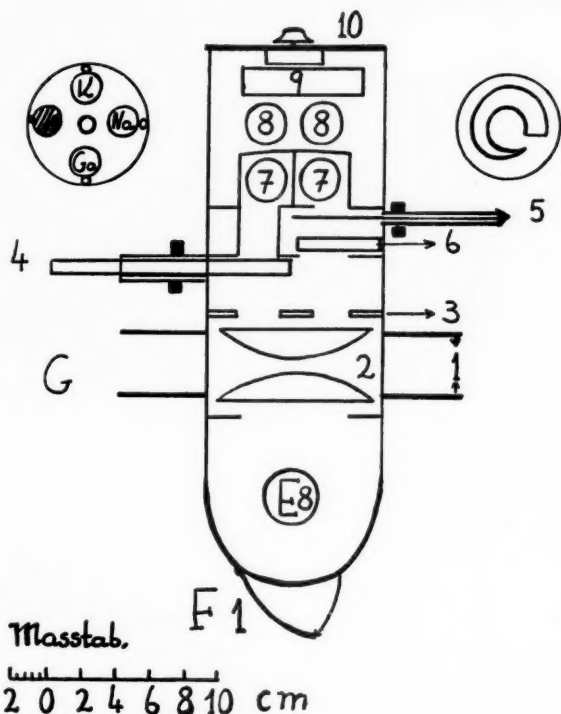


Abb. 3. — Optisches System im Nebenrohr des Flammenphotometers<sup>1</sup> (vgl. Abb. 2 Teil G.).

7. *Der Verstärker.* — Der Verstärker wurde von einem von uns (Mettinen) gebaut. Der Bau desselben geht aus dem Schaltungs-schemata Abb. 4 und 5 hervor. Der grösste Teil der Verstärkereinheiten mit allen dazugehörigen Trockenelementen ist in einen getrennten »Kontrollkasten« aus Blech von  $215 \times 145 \times 160$  mm Grösse eingebaut.

8. *Galvanometer.* — Als Galvanometer wurde der von der Firma Dr. B. Lange, Berlin-Zehlendorf, gelieferte hochempfindliche Multi-flex-Galvanometer, Type MGF 2 (Empfindlichkeit  $3 \cdot 10^{-9}$  A/mm) benutzt. Als Skala wurde eine 200 mm lange Lichtzeigerskala verwendet. Das Instrument besitzt für Messungen in verschiedenen Konzentrationen drei umschaltbare Messbereiche. Für Blutanalysen wurde ausschliesslich der Messbereich 1 mit Galvanometerdämpfung (Eingangswiderstand 1110 Ohm) benutzt. Der zu dem Galvanometer

<sup>1</sup> Erklärungen gegeben in Abb. 2.

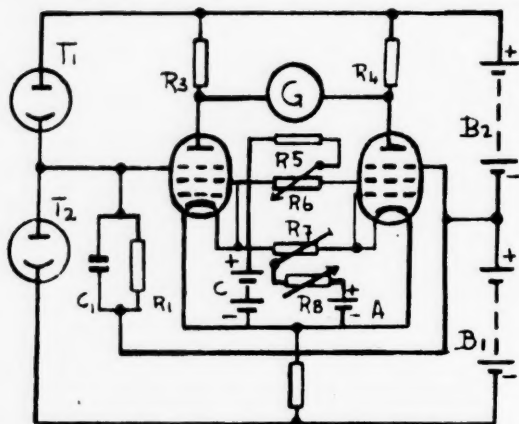


Abb. 4. — Vereinfachtes Schema des gesamten elektrischen Systems.

*Erklärung der Teile:**Widerstände*

$R_1 = 200 \cdot 10^6$	Ohm	spezial
$R_2 = 220\,000$	„	$\frac{1}{4}$ W 10 % Kohle
$R_3 = 200\,000$	„	1 „ Hohe Stabilität
$R_4 = 200\,000$	„	1 „ geeicht gegeneinander
$R_5 = 25\,000$	„	1 „ 10 % Kohle
$R_6 = 10\,000$	„	3 „ Spulenpotentiometer
$R_7 = 10$	„	3 „ „
$R_8 = 0 \dots 5$	„	3 „ Spulenrheostat
$R_9 = 1\,000\,000$	„	1 „ Hohe Stabilität

*Kondensatoren*

$C_1 = 2\,000$	pF	versilb. Mica, geringer Stromverlust (Leakage)
$C_2 = C_3 = 0.5$	F	500 V, Filterkondensatoren
$C_4 = C_5 = 0.005$	„	„

*Induktoren*

$L_1 = L_2 = 2 \frac{1}{2}$	mH	R.F.C. Drosselspulen
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*Elektronenröhren*

$T_1 =$	Vacuumphotozelle	90 CV für Kalium und 90 AV für Natrium
$T_2 =$	„	90 CV für Lithium
$T_3 = T_4 =$	DK 40	geeichtes Paar (Philips)

*Trockenelemente*

$A = 1 \frac{1}{2}$	V	grosse Kapazität, für die Glühfäden der Röhren $T_3$ & $T_4$
$C = 9$	V	grosse Kapazität, Gitterspannungselement
$B_1 = B_2 = 22 \frac{1}{2}$	V	Messungselement

*Weitere Teile*

mA	Milliampèremeter	0—100 oder 0—200 mA
G	Galvanometer	
O	Oktalkontakt	
S	3-poliger Ein- und Ausschalter	
$S_c$	150 cm langer Kabel	mit 8 Leitungen. Kabel mit Metallschutz versehen, der die Endplatte des Flammenphotometers mit Knopf 2 des Oktalsockels verbindet (Erdleitung).

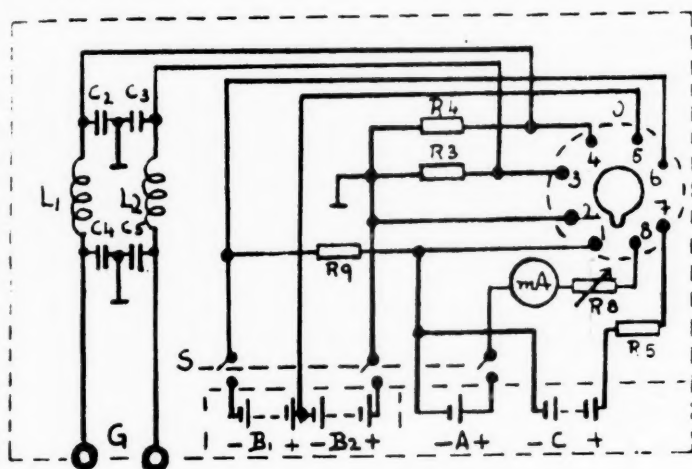


Abb. 5. — Schaltungsdiagramm der Batterien und des Kontrollkastens (vgl. die Erklärung der Teile von Abb. 4).

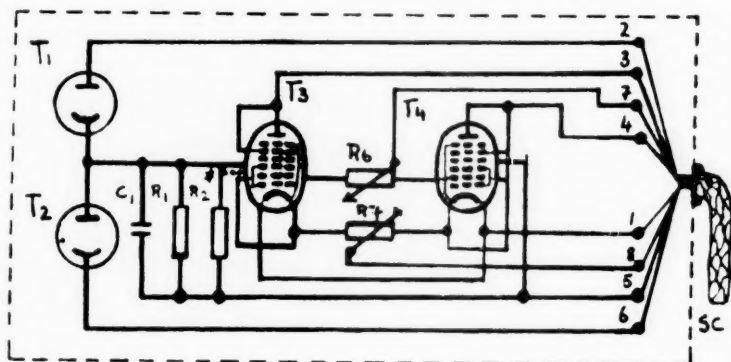


Abb. 6. — Schaltungsdiagramm des elektrischen Systems in der Nebenröhre des Flammphotometers (Photozellen- und Verstärkereinheit, vgl. die Erklärung der Teile von Abb. 4).

\* = Freies Ende von  $R_2$  verbunden mit Kontakt Nr. 7, N.C. an dem Sockel von  $T_3$  zur Ausbalanzierung der Leakageströme von Kontakt Nr. 5 (ca. + 20 V) zu Kontakt Nr. 6 (Kontrollgitter mit hoher Impedance)

**Wichtig:** Die Elemente A und C müssen gut isoliert sein von einander und von dem Verstärkerkasten.

gehörende Vorschaltkasten mit zwei Regelwiderständen zur kontinuierlichen Empfindlichkeitsreglung wurde umgebaut, anstatt der Kohlenwiderstände wurden Spulenwiderstände verwendet.

*Das optische System.* — Die Anordnung des optischen Systems geht aus der schematischen Abbildung (Abb. 3) hervor. Im Nebenrohr des Flammenphotometers ist eine grosse Kondensorlinse angebracht, wie in dem Apparat von Dr. Lange. Der Abstand der Linse von der Flamme ist so eingestellt, dass der stabilste und heisseste Teil der Flamme im Brennpunkt liegt. Vor der Linse ist eine Asbestblende mit einer 35 mm breiten und 18 mm hohen Öffnung angebracht. Diese Blende verhindert, dass zuviel Licht auf die Photozellen fällt und diese mit Licht übersättigt werden. Das Licht wird in der Kondensorlinse parallelgerichtet. Hinter der Kondensorlinse befindet sich in dem Nebenrohr ein metallener Fensterrahmen mit zwei Fensteröffnungen, der die Lichtstrahlen in zwei gleichgrosse Lichtbündel teilt. Die Fensteröffnungen sind 30 mm hoch und 24 mm breit. Das linke Lichtbündel geht dann durch die auswechselbaren Filter der Drehscheibe, das rechte dagegen durch das feste Lithiumfilter (6709 Å). Als Filter werden die von Dr. B. Lange gelieferten Interferenzfilter verwendet, die für scharf abgegrenzte Wellenlängen angefertigt werden und sehr selektiv sind. Auf der Lithiumseite befindet sich vor der Photozelle eine drehbare Blenderscheibe, mit einer schlitzförmigen Öffnung, die nach der einen Seite hin immer schmaler wird und zur Regulierung der Grösse des Lichtbündels dient. Die Photozellen sind rot- bzw. blausensitive Vacuumphotozellen, die in einen speziellen, lichtdichten Behälter einmontiert sind. Auf der Li-Seite wird immer eine rotsensitive Zelle (Mullard 90 CV) verwendet, während auf der Analysenseite sowohl eine rotsensitive für Kaliumbestimmungen (Mullard 90 CV) als auch eine blausensitive für Natriumbestimmungen (Mullard 90 AV) eingesetzt werden kann. Die Interferenzfilter für Natrium (5890 Å), Magnesium (5530 Å) und Calcium (6160 Å) lassen die Kaliumemission durch. Aus diesem Grunde muss bei Natriumbestimmungen eine blausensitive Photozelle verwendet werden, welche den roten Teil des Spektrums weglässt, wie aus Abb. 7 zu entnehmen ist. — Hinter den Photozellenbehältern befindet sich auf jeder Seite eine Elektronenröhre. Auf der Verschlusscheibe aus Pakelit ist der Regulationswiderstand des Dunkelstroms angebracht. Das Licht für beide Photozellen kommt bei diesem Apparat von derselben Seite

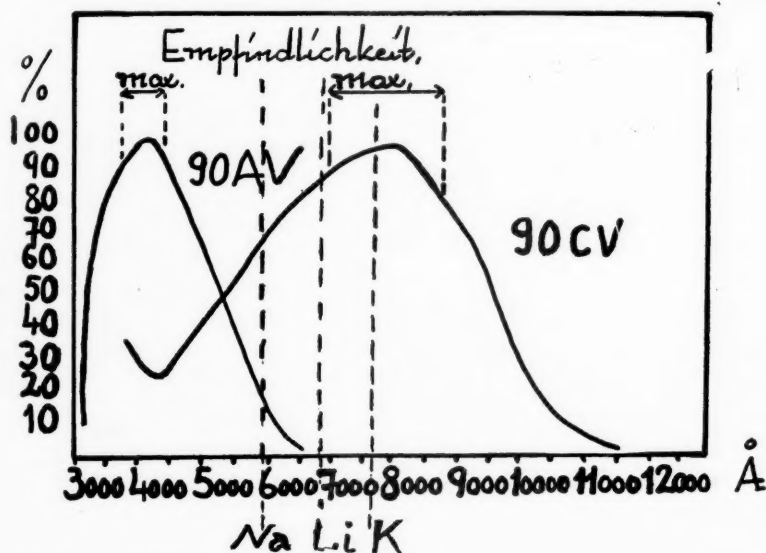


Abb. 7. — Relative Empfindlichkeitskurven der Vacuum-Photozellen (nach den Fabriksangaben, Mullard Electronic Products LTD. Lond.).

der Flamme, sodass das Emissionslicht auf beiden Seiten praktisch das Gleiche ist. Hinter den Photozellenbehältern befindet sich ferner noch eine kleine Schachtel mit Blaugel zur Trockenhaltung des Systems.

*Das elektrische System.* — Die Einzelheiten der Schaltung gehen aus den Abb. 4—6 hervor. Der Galvanometer gibt schon nach 0.4 Sek. den maximalen Ausschlag, da der innere Widerstand der Messröhre  $R_g = 200 \cdot 10^6$  Ohm beträgt und die Gitterkapazität  $C_g = 2000 \cdot 10^{-12}$  F ist. Hieraus ergibt sich für die Zeitkonstante des Richtungsgitterkreises  $\tau = R_g \cdot C_g [\text{s}] = 200 \cdot 10^6 \cdot 2000 \cdot 10^{-12} = 0.4$  Sek.

#### VORBEREITUNG DES FLAMMENPHOTOMETERS UND AUSFÜHRUNG DER BESTIMMUNGEN

*Blutproben.* — Das Venenblut wird in gewöhnliche, ca 12 ml fassende Zentrifugenröhren genommen, in denen sich 1 mg trockenes und reinstes Heparin befindet. Da das gewöhnliche Heparin in 5% Lösung (Heparin Medica) etwas mehr Alkalimetalle enthält als andere Heparinpräparate, wie aus den Versuchen in Tabelle 1 zu

entnehmen ist, wurde pulverisiertes, angeblich Natrium-freies Heparin verwendet (Anachemia LTD, Montreal). Von diesem Präparat wird am vorteilhaftesten eine 0.5% Lösung angefertigt und 0.2 ml (= 1 mg) in jede Zentrifugenröhre pipettiert. Die Zentrifugenröhren werden dann zum Abdunsten der Flüssigkeit eine zeitlang in einem Thermostat von 37° C gehalten. Die Röhren enthalten nun alle genau 1 mg Heparin, das für 10 ml Blut ausreicht. Es ist angebracht jedesmal genau 10 ml Blut mit einer Rekordspritze zu entnehmen, um den Hepariningehalt in allen Proben möglichst konstant zu halten. Die Blutprobe in der Zentrifugenröhre ist sofort mehrmals gegen den Gummipropfen zu mischen um jegliche Gerinnung zu vermeiden.

Die Proben sollen dann unmittelbar zentrifugiert werden um eine eventuelle Diffusion von Kalium aus den roten Blutkörperchen in das Plasma zu verhindern. Man zentrifugiert zweimal, das erste Mal 30 Minuten mit 3.500 Umdrehungen per Minute, wonach das Plasma möglichst akkurat abpipettiert wird. Sowohl das Plasma als auch die roten Blutkörperchen werden dann von neuem 15 Minuten mit derselben Geschwindigkeit zentrifugiert, wonach das Plasma nochmals von eventuellen Blutzellenresten abpipettiert wird und die Blutzellen andererseits wiederum möglichst genau von dem überstehenden Plasma befreit werden. Zwischen den Spalten der Erythrozyten bleibt natürlich immer eine geringe Quantität Plasma zurück, welche einen Fehler verursacht bei der Natrium- bzw. Kaliumbestimmung der roten Blutkörperchen. Dieser Fehler dürfte aber bei Einhaltung der obengenannten Vorschriften 3—5% nicht überschreiten, wie aus Untersuchungen von zweien von uns (Krusius und Leppänen), die an anderer Stelle publiziert werden, hervorgeht. Von dem Plasma und den Blutzellen werden dann separate Verdünnungen für die Natrium- und Kaliumbestimmung hergestellt. Die Verdünnungen müssen so berechnet werden, dass die ungefähre Konzentration für Kaliumbestimmungen zwischen 0.05 und 0.30 m.eq./L fällt und für Natrium entweder zwischen 0.05 bis 0.30 oder 0.10 bis 0.40 m.eq./L. Von jeder Blutprobe sind daher vier Verdünnungen herzustellen: 1. (vgl. Schema): Plasma Kalium: 2 ml Plasma ad 50 ml in einer Massflasche (Verd. 4: 100, Verdünnungsfaktor 25), 2. Erythrozyten Kalium: 2 ml Blutkörperchen ad 100 ml aq. redest. in einer Massflasche, von der nach Hemolyse der Blutkörperchen 5 ml ad 50 ml verdünnt werden (Verd. 0.2: 100, Verdün-

VERDÜNNUNGSSCHEMA DER BLUTPROBEN

	Kalium	Natrium
Plasma	4:100 F=25	0.2:100 F=500
Blutkörper	0.2:100 F=500	2:100 1:100 F=50 F=100
Vollblut	0.4:100 F=250	0.2:100 F=500

nungsfaktor 500) 3. Plasma Natrium: 0.1 ml Plasma ad 50 ml (Verd. 0.2: 100, Verdünnungsfaktor 500) und 4. Erythrozyten Natrium: 2 ml Blutkörperchen ad 100 ml aq.redest. (wie unter 2) und von dieser Lösung 25 ml ad 50 ml (Verd. 1: 100, Verdünnungsfaktor 100). oder 2 ml Blutkörper ad 50 ml aqua und davon 25 ml ad 50 ml (F=50). — Jede endgültige Verdünnung soll 7.5 m.mol Lithiumsulfat ( $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$ ) enthalten; daher gibt man am einfachsten in die endgültigen Verdünnungsmassflaschen immer das halbe Volumen 15 m.mol Lithiumsulfatlösung und füllt zuletzt bis zur Marke mit redestilliertem Wasser. Zum Verhindern des Schäumens der Verdünnungen hat es sich als vorteilhaft erwiesen einen kleinen Tropfen reinsten Oktylalkohols hinzuzugeben. Will man auch Bestimmungen im Vollblut ausführen, müssen vor dem Zentrifugieren noch zwei weitere Verdünnungen gemacht werden: 2 ml Vollblut werden mit aq.redest. auf 100 ml verdünnt und hemolysiert. Von dieser Verdünnung nimmt man für Kaliumbestimmungen 10 ml ad 50 ml (Verd. 0.4: 100, Verdünnungsfaktor 250) und für Natriumbestimmungen 5 ml ad 50 ml (0.2: 100, Verdünnungsfaktor 500). Auch zu diesen Verdünnungen wird natürlich das halbe Volumen der 15 m.mol Li-Lösung gegeben.

Eingehende Versuche haben ergeben, dass eine Ausfällung der Bluteiweisskörper unnötig ist. Von allen versuchten Fällungsmethoden erwies sich die Ausfällung mit Salpetersäure als am geeignetsten, im allgemeinen war aber die Ausbeute etwas kleiner als ohne Ausfällung. Trichloressigsäurefällung kann bei flammenphotometrischen Bestimmungen nicht benutzt werden, ebenso sind alle, Alkalimetalle enthaltende Fällmittel ungeeignet. Auch manche Schwermetallsalze können stören bei den Bestimmungen. Das Weg-

lassen der Eiweissfällung macht die Ausführung der Analysen einfacher und die Methode gewinnt dabei an Genauigkeit.

Bei den Natriumbestimmungen ist die Reinigung der Glasgefässe, Pipetten etc. mit besonders grosser Sorgfalt auszuführen, da man sonst erstaunlich grosse Fehler erhalten kann. Es empfiehlt sich alles Glasgerät nach gewöhnlichen Abwaschen für 12 Stunden in Bichromat-Schwefelsäure aufzubewahren und danach reichlich mit Wasser, zuletzt mit redestilliertem Wasser, zu spülen und im Wärmeschrank zu trocknen. Ferner sollen die Natriumbestimmungen möglichst schnell ausgeführt werden, um eine Natriumabgabe aus dem Glas zu vermeiden. Zur weiteren Ausschaltung von Fehlern bei der Bereitung der Verdünnungen, sollten immer dieselben Normalpipetten verwendet werden.

*Urinproben.* — Die Harnverdünnungen werden gemäss demselben Prinzip gemacht, auch hier ist die endgültige Lithiumkonzentration immer 7.5 m.mol Lithiumsulfat. Gewöhnlich bedarf man 0.2—2.0 ml Harn, jenachdem wieviel Alkalimetalle die zu untersuchende Probe enthält. Es empfiehlt sich daher gleich mehrere Verdünnungen anzufertigen.

*Standardlösungen.* — Als Stammlösungen verfertigt man am vorteilhaftesten eine 100 m.eq./L Lösung von Natriumchlorid (NaCl p.an. im Wärmeschrank getrocknet, 5.845 g ad 1000 ml) und Kaliumchlorid (KCl p.an., getrocknet, 7.456 g ad 1000 ml). Von diesen Stammlösungen werden dann die erforderlichen Standardlösungen bereitet. Für Kaliumbestimmungen 0.05, 0.075, 0.10, 0.13, 0.17, 0.20, 0.23, 0.27, und 0.30 m.eq./L und für Natrium 0.05 oder 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 und 0.40 m.eq./L. — Diese Lösungen enthalten wiederum 7.5 m.ml. Lithiumsulfat. Die Natriumstandardlösungen sollen in mit z.Bsp. Ceresinwachs bedeckten Flaschen aufbewahrt werden um eine Lösung des Natriums aus dem Glas zu vermeiden. Die Lithiumkonzentration von 7.5 m.mol erwies sich auf Grund von längeren Versuchen als am günstigsten. Im Prinzip ist es wichtig, dass die Kompensationsstelle stabil ist und dass die Ablesungen der aufeinanderfolgenden Standardlösungen genügend grossen Unterschiede aufweisen. Alle Standardlösungen sind natürlich jedesmal neu herzustellen, wenn eine neue Lithiumsulfatlösung in Gebrauch genommen wird. Bei den verwendeten starken Blut- bzw. Plasmaverdünnungen der Analysen (vgl. Verdünnungsschema) verursacht die Viskosität der Proben keinen Fehler; ein Zusatz von

Protein, Cholesterol etc. zu den Standardlösungen erübrigt sich daher. Auch dies vereinfacht den Arbeitsgang bedeutend.

*Inbetriebsetzung des Flammenphotometers.* — Der Flammenphotometer soll ca 10—15 Minuten vor Ausführung der Bestimmungen in Betrieb gesetzt werden. Dann nimmt man die Nulleinstellung des Multiflex-Galvanometers vor. Hierzu muss der Galvanometer von dem Stromkreis des Flammenphotometers getrennt werden, wonach der Nullpunkt auf der Skala mit der Grob- bzw. Feineinstellschraube eingestellt wird. Dann wird der Galvanometer wieder in den Stromkreis eingeschaltet und die Öffnungen zu den Photozellen werden verschlossen. Auf der Lithiumseite wird dieses durch Schliessen der Blende erzielt, auf der anderen wiederum durch Verschieben einer schwarzen Scheibe, die in einer Öffnung der Filterdrehscheibe angebracht ist. Hiernach wird der ganze Apparat mittels des Dunkelstroms auf 0 eingestellt. Nach Einstellung des Dunkelstroms werden die Öffnungen der Photozellen wieder freigemacht und links das jeweils erforderliche Filter vorgeschoben. Bei Kaliumbestimmungen stellt man dann den Galvanometer auf 0 mit der 0.05 m.eq./L Standardlösung durch Einstellung der Blendenöffnung. Hiernach wird mit der Standardlösung 0.30 m.eq./L unter Einstellung der Empfindlichkeit des Vorschaltkastens (Grob- bzw. Feineinstellung) der Endpunkt auf der rechten Seite der Skala (200 mm) gesucht. Dann wird die 0-einstellung noch einmal mit der 0.05 m.eq./L. Lösung kontrolliert. Bei Natriumbestimmungen verfährt man analog: Für den 0-punkt verwendet man entweder die 0.05 m.eq./L Lösung oder, was uns praktischer scheint, die 0.10 m.eq./L Lösung. Der 200-Punkt wird dann entweder mit der 0.30 oder der 0.40 m.eq./L Lösung eingestellt.

Die farblose Leuchtgasflamme ruft schon allein eine bestimmte Abweichung vom 0-punkt hervor, welche als Streulicht bezeichnet wird. Das Streulicht darf sich während den Bestimmungen nicht verändern, da sich sonst auch die Standardkurve verändert. Es soll daher immer ab und zu kontrolliert werden. Gewöhnlich beträgt das Streulicht in unserem Apparat bei Kaliumbestimmungen 9—14 mm und bei Natriumbestimmungen 0—1 mm.

Nach Einstellung des Apparates wird eine Ablesung mit jeder einzelnen Standardlösung vorgenommen zur Bestimmung der Standardkurve (Abb. 8). Diese verläuft nahezu linear und ist an verschiedenen Bestimmungstagen bei gleicher Einstellung des Appara-

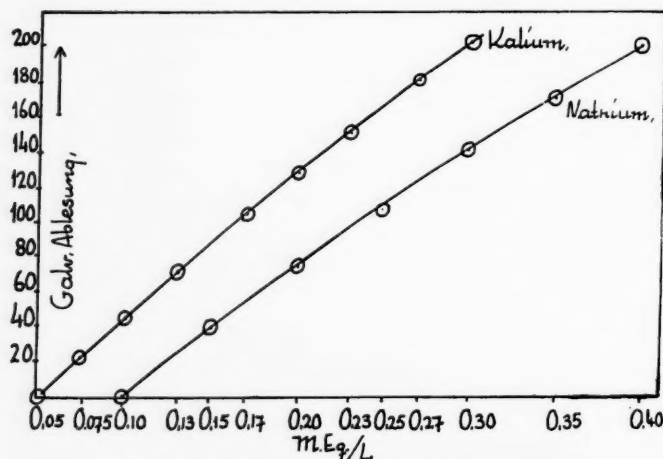


Abb. 8. — Typische Kalium- und Natriumstandartkurve.

tes so gut wie identisch. Nach Anlegung der Standartkurve werden die Analysen abgelesen. Es empfiehlt sich bei längeren Serienbestimmungen die Kurve ab und zu während des Arbeitsganges mit einigen Standartlösungen zu kontrollieren. Auch sollte man der Kontrolle halber sowohl die Standartkurve als auch die Analysen zweimal ablesen. Der Luftdruck wird während der Bestimmung konstant auf 0.6 atü gehalten und der Gasdruck auf ca 40 mm Wasser. Nach Verneblung einer jeden Probe soll die Kammer durch Verneblung von redestilliertem Wasser gespült werden.

#### GENAUIGKEIT DER METHODE

Der Apparat ist schon seit über einem Jahr im Routinegebrauch des Laboratoriums und hat zur völligen Zufriedenheit funktioniert. Im folgenden sollen einige Kontrolluntersuchungen der Methode und der Apparatur kurz besprochen werden. In Tabelle 1 sind einige Analysen von Heparinpräparaten angeführt. Aus diesen Untersuchungen geht hervor, dass keins der geprüften Heparinpräparate in den von uns verwendeten Verdünnungen bei den Kalium- oder Natriumbestimmungen einen beachtenswerten Fehler hervorrufen kann. Am wenigsten Kalium und auch Natrium enthielt das Präparat I von Anachemia LTD, Montreal, aber auch das in der Behandlung von Tromboembolien in unserer Klinik am meisten

TABELLE 1

NATRIUM- UND KALIUMGEHALT VERSCHIEDENER HEPARINPRÄPARATE

Heparinpräparat	1 mg Heparin enthält		Heparinfehler in m.eq./L	
	Natrium mg	Kalium mg	Natriumbest. in Plasma u. Erythrozyten	Kaliumbest. in Plasma u. Erythrozyten
Anachemia LTD Präp. I	0.0998	0.000215	+0.434	+0.00055
Anachemia LTD Präp. II	0.1391	0.000293	+0.605	+0.00075
Medica, Farm. Fabr. Finnland .....	0.1518	0.002737	+0.660	+0.00700

benutzte Präparat von Medica (5% Heparinlösung zur Injektion) enthielt nicht soviel Kalium bzw. Natrium, dass es bei der Flammenphotometrie einen beachtenswerten Fehler verursachen kann.

Die Ausbeuteversuche nach Zusatz einer Kalium- bzw. Natriumlösung zu Blut- bzw. Plasmaverdünnungen haben ein zufriedenstellendes Resultat ergeben (Tab. 2 und 3). Die Kaliumausbeute nach Zusatz von 100 mikroeq./L Kalium zu Vollblutverdünnungen war sehr gut und variierte der Fehler bei 10 Bestimmungen zwischen  $-0.6$  und  $+3.0\%$ . Durchschnittlich war die Ausbeute etwas zu gross ( $+0.69\%$ ). Der Fehler bei den Ausbeuteversuchen nach Zusatz von 100 mikroeq./L Natrium zu Vollblut- bzw. Plasmaverdünnungen (Tab. 3) war etwas grösser und schwankte zwischen  $-6.4$  und  $+4.4\%$ . Der Durchschnittsfehler von 15 Parallelbestimmungen betrug  $+0.57\%$ .

Auf Grund der Ausbeuteversuche lässt sich sagen, dass der Fehler bei den Kaliumbestimmungen so geringfügig ist, dass er in der Klinik keine Rolle spielt. Hauptsächlich dürfte unseres Erachtens der Fehler als Pipettierungs- und Verdünnungsfehler zu erklären sein und könnte wahrscheinlich durch Anwendung von kontrollierten Normalmassflaschen und -Pipetten stark reduziert werden. Ein gewisser Galvanometer-Ablesungsfehler muss aber immer verbleiben, da bei einem so empfindlichen System der Lichtzeiger nicht ganz still steht und man bei den Alesungen einen Mittelwert der Schwankungen nach beiden Seiten zu nehmen hat. Aus diesem Grunde empfiehlt es sich, wie schon erwähnt, mehrere Ableseungen jeder Probe ebenso wie der Standardlösungen vorzunehmen. Der grössere Fehler bei den Natriumbestimmungen dürfte haupt-

TABELLE 2

KALIUMAUSBEUTE NACH ZUSATZ VON 100 MIKROEQ./L (= 0.10 M.EQ./L) ZU VOLLBLUTVERDÜNNUNGEN

Vollblut <sup>1</sup> mikroeq./L	Ausbeute <sup>1</sup>		
	Gefunden mikroeq./L	Berechnet mikroeq./L	% Fehler
91	190	191	99.5
70	171	170	100.6
54	153	154	99.4
90	190	190	100.0
56	158	156	101.3
86	188	186	101.1
98	204	198	103.0
67	168	167	100.6
90	193	190	101.6
79	179	179	100.0

TABELLE 3

NATRIUMAUSBEUTE NACH ZUSATZ VON 100 MIKROEQ./L (= 0.10 M.EQ./L) ZU VOLLBLUT- BZW. PLASMAVERDÜNNUNGEN

Vollblut bzw. <sup>1</sup> Plasma mikroeq./L	Ausbeute <sup>1</sup>		
	Gefunden mikroeq./L	Berechnet mikroeq./L	% Fehler
78	180	178	101.1
108	196	208	94.2
126	235	226	104.0
155	266	255	104.4
196	295	296	99.7
102	203	202	100.5
124	231	224	103.1
154	258	254	101.6
193	291	293	99.7
256	354	256	99.4
150	252	250	100.8
151	235	251	93.6
209	320	309	103.6
122	223	222	100.5
126	231	226	102.2

<sup>1</sup> Mittelwert aus zwei Parallellablesungen

TABELLE 4

PARALELLBESTIMMUNGEN IM SERUM MIT DER TITRIMETRISCHEN METHODE VON KRAEMER &amp; TISDALL

Flammenphotometer mg %	Kraemer & Tisdall mg %	Prozentueller Unterschied vom Flammenphotometerwert
19.1	17.9	— 6.3
16.8	16.9	+ 0.6
20.5	18.3	—10.7
18.3	16.2	—11.5
19.1	18.1	— 5.2
17.8	17.7	— 0.6
20.8	19.2	— 7.7
18.9	18.4	— 2.6
17.6	15.3	—13.1
22.5	23.3	+ 3.5
17.4	16.2	— 6.9
19.9	18.5	— 7.0
21.3	17.8	—16.4
17.8	15.9	—10.7
$\Delta$ 19.09	$\Delta$ 17.83	$\Delta$ — 6.75

sächlichst dadurch bedingt sein, dass der Ablesungsfehler hier wegen etwas grösserer Schwankungen des Lichtzeigers grösser ist. Daneben ist aber nicht zu vergessen, dass ein gewisser Glassfehler hinzukommt, der durch den Natriumgehalt der Glasswaren bedingt wird und von der Qualität der Glassgefässe sowie von der Zeit abhängt, die von Entnahme der Blutproben bis zur Galvanometerablesung verfliesst. In der Regel sollten die Natriumbestimmungen immer sobald als möglich ausgeführt werden und wenigstens am selben Tage. Bei den Kaliumbestimmungen kann man dagegen die Verdünnungen gut stehen lassen. Es ist möglich, dass es vorteilhaft wäre für die Natriumbestimmungen ein Spezialglas zu verwenden.

In Tabelle 4 sind 14 Paralellbestimmungen im Serum mit dem Flammenphotometer und der titrimetrischen Methode von Kraemer-Tisdall (2) angeführt. Die Übereinstimmung ist nicht sehr gut, der prozentuelle Fehler bezogen auf den Flammenphotometerwert, schwankte zwischen —16.4 und +3.5, im allgemeinen waren die titrimetrischen Kaliumwerte bedeutend niedriger, durchschnittlich —6.75%. Nur in zwei Fällen war der titrimetrische Wert grösser als

TABELLE 5

VERGLEICHSBESTIMMUNGEN MIT DEM FLAMMENPHOTOMETER IM PLASMA UND DER TITRIMETRISCHEN METHODE IN SERUM

Flammenphotometer Plasma mg %	Titrimetrische Methode Serum mg %	Prozentueller Unterschied vom Plasmawert
18.4	17.9	+ 2.6
16.5	16.9	— 2.4
19.4	18.3	+ 5.7
17.9	16.2	+ 9.5
19.9	18.1	+ 9.1
17.6	17.7	— 0.6
19.9	19.2	+ 3.5
18.2	18.4	— 1.1
17.1	15.3	+10.5
17.8	23.3	—28.1
15.0	16.2	— 8.0
17.7	18.5	— 4.5
19.9	17.8	+10.5
16.8	15.9	+ 5.4
17.8	17.1	+ 3.9
18.5	18.1	+ 2.2
21.7	21.0	+ 3.2
16.4	16.1	+ 1.8
18.0	17.2	+ 4.4
19.2	16.7	+13.0
18.3	14.9	+18.5
17.8	22.0	—23.6
18.8	20.8	—11.1
17.6	18.9	—7.4
15.4	19.7	—27.9
$\Delta$ 18.06	$\Delta$ 18.09	$\Delta$ — 0.4

der Flammenphotometerwert. Die grosse Diskrepanz zwischen beiden Methoden dürfte durch die Ungenauigkeit der titrimetrischen Methode zu erklären sein, vor allem durch den schwankenden Kaliumgehalt des ausgefällten Kalium-Koboltnitrit-komplexes.

In Tabelle 5 sind ferner 25 Parallellbestimmungen zwischen dem Flammenphotometer und der titrimetrischen Methode angeführt, hier sind aber die Flammenphotometerbestimmungen im Plasma ausgeführt, während die titrimetrischen Bestimmungen im Serum vorgenommen wurden. Die Schwankung des prozentuellen Fehlers ist bei diesen Bestimmungen noch viel grösser als in der Tabelle 4

TABELLE 6

VERGLEICHSBESTIMMUNGEN IN PLASMA UND SERUM MIT DEM FLAMMENPHOTOMETER

Plasma mg %	Serum mg %	Prozentueller Unterschied vom Plasmawert
18.4	19.1	+ 3.8
16.5	16.8	+ 1.8
19.4	20.5	+ 5.7
17.9	18.3	+ 2.2
19.9	19.1	— 4.0
17.6	17.8	+ 1.1
19.9	20.8	+ 4.5
18.2	18.9	+ 3.8
17.1	17.6	+ 2.9
17.8	22.5	+26.4
15.0	17.4	+16.0
17.7	19.9	+12.4
19.9	21.3	+ 7.0
16.8	17.8	+ 5.4
17.9	20.1	+12.3
$\Delta$ 18.00	$\Delta$ 19.19	$\Delta$ + 6.75

(—28.1 u. +18.5%), jedoch ist der Durchschnittswert der Bestimmungen mit beiden Methoden hier der Gleiche. Da die Flammenphotometerwerte in Tabelle 4 durchschnittlich deutlich höher liegen als die entsprechenden titrimetrischen Werte deuten die Ergebnisse der Bestimmungen in Tabelle 5 daraufhin, dass die Kaliumwerte in Serum höher liegen als im Plasma. Um diesen Befund zu sichern wurden ferner flammenphotometrische Parallelb Bestimmungen in Plasma und Serum ausgeführt (Tabelle 6). Bei diesen 15 Vergleichsbestimmungen lag der Serumwert durchschnittlich deutlich höher (+6.75%). Der prozentuelle Unterschied zum Plasma schwankte zwischen —4% und +26.4%. Alle Serumwerte mit Ausnahme von einem waren grösser als die Plasmawerte. Für die höheren Kaliumwerte im Serum gibt es mehrere Erklärungsmöglichkeiten, am wahrscheinlichsten scheint es uns aber, dass bei der Gerinnung des Blutes eine gewisse Hemolysetendenz der roten Blutkörperchen bestehen kann, die zu einem Austritt von Zellkalium in das Serum führt. Hierfür spricht auch die Tatsache, dass der prozentuelle Unterschied zwischen Plasma- und Serumkalium nicht konstant ist sondern grossen Schwankungen unterliegt. — Die Bestimmungen

in Tabelle 5 und 6 zeigen daher, dass es richtiger ist, den Kaliumgehalt des Plasmas als des Serums zu analysieren. In anderem Zusammenhange soll von zweien von uns (Krusius und Leppänen) über Vergleichsbestimmungen des Kaliumgehalts in Erythrozyten, Vollblut und Plasma berichtet werden.

#### ZUSAMMENFASSUNG

Es wird ein neuer, einfacher und akurater Flammenphotometer beschrieben, der mit zwei Photozellen arbeitet, von denen die eine als Kompensationszelle dient, während die andere die eigentliche Messung ausführt. Der Apparat arbeitet mit einer stabilen Leuchtgasflamme, deren Emission durch zwei Interferenzfilter monochromatisiert und von den Photozellen registriert wird. Die erhaltenen elektrischen Impulse werden gegeneinander kompensiert und die entstandene Differenz wird durch einen Verstärker verstärkt und mit einem empfindlichen Galvanometer gemessen. Eine Drehkolben-Vakuumpumpe liefert den nötigen Druck zur Verneblung der Flüssigkeit im Vernebler. Der Luftdruck und Gasdruck wird durch Ventile reguliert. Der Apparat eignet sich zu Kalium- und Natriumbestimmungen in Blutplasma, Serum, Vollblut und Erythrozyten, aber auch zur Analyse anderer biologischer Flüssigkeiten. An Hand von Ausbeuteversuchen und Kontrollbestimmungen in Plasma und Serum wird die Genauigkeit der Analysenresultate gezeigt.

#### SCHRIFTTUM

1. HALLMAN, NILO UND LEPPÄNEN, VEIKKO: *Ann. Med. Int. Fenn.* 1952:41:21.
  2. KRAMER, B. UND TISDALL, F. F.: *J. Biol. Chem.* 1921:46:339; 1921:48:223.
  3. LEPPÄNEN, VEIKKO UND FORSANDER, O.: *Scand. J. Clin. a. Lab. Invest.* 1951:3:33.
  4. RIEHM, H.: *Z. anal. Chem.* 1948:128:249.
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CHROMOSOME NUMBER AND ANTI-A1-AGGLUTININS IN SEEDS OF *VICIA CRACCA*

by

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(Received for publication August 2, 1952)

After the first report dealing with the finding of specific agglutinins in the seeds of *Vicia Cracca* and *Cytisus sessilifolius* (1) some negative observations have also been published. Thus Grubb found no anti-O-agglutinins in seeds of *Cytisus sessilifolius* grown in the Lund Botanical Garden (2) and Krüpe could not observe anti-A1-agglutinins in seeds of *Vicia Cracca* v. *Gerardi* (3).

In order to study any divergencies we obtained 12 samples of seeds of *Vicia Cracca* from different parts of Europe and one from Manitoba, Canada. Most of them derived from university botanical gardens.

From these seeds saline extracts (1:20) were prepared. The extracts were titrated with A1, A2, B, A1B, A2B and O cells. The technique was the same as used in the earlier study (1). — The number of chromosomes was counted from the growing root tips. In addition, the number of seeds in 200 mg was counted.

The results were as follows:

Number of Samples	Chromosome Number (2 n)	Number of Seeds in 200 mg	Origin of Samples
GROUP I. SEEDS CONTAINING A STRONG ANTI-A1-AGGLUTININ			
7	28	14—16	Copenhagen, Dijon, Edinburgh, Cologne, Lippstadt, Manitoba, Vienna
1	28	32	Iceland
1	27	14	Berlin
1	14	22	Bydgoszcz, Poland
GROUP II. SEEDS CONTAINING AGGLUTININS WITH NO SPECIFICITY			
1	24	6	Brussels
2	12	6,9	Both from Kew Gardens, Ld

The main rule seems apparent to us. Most of the samples (9/13) contained the regular anti-A1-agglutinins. The number of chromosomes in this group was 28 and in one sample half of it, or 14. Besides these there was a small group of seeds with no specific agglutinins, which had 24 or 12 chromosomes and were distinctly larger in size. The Chromosome numbers of 28, 14 and 12 are previously known.

In addition there was one peculiar sample from Berlin with 27 chromosomes. It will deserve further study and may be left without comment here.

It seems, thus, as if the serological qualities of *Vicia Cracca* depend on the number of chromosomes in the plant.

#### REFERENCES

1. RENKONEN, K. O.: Ann. Med. Exp. et Biol. Fenn. 1948:26:66.
2. GRUBB, R.: Acta Path. et Microbiol. Scand. 1949:suppl. 83.
3. KRÜPE, M.: Z. f. Immunit.forsch. 1950:107:450.

## STUDIES ON THE VITAMIN B<sub>12</sub> CONTENT OF HUMAN BLOOD PLASMA

### PRELIMINARY REPORT

by

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(Received for publication August 15, 1952)

Reports in the literature on the vitamin B<sub>12</sub> content of blood and blood plasma are very few and so far highly contradictory. Ross (4) reported that using Euglena method he had obtained 185 m $\mu$ g of this vitamin per millilitre of plasma, and that when diluted blood plasma was heated the vitamin B<sub>12</sub> bound to the proteins was released and he was able to demonstrate 450—500 m $\mu$ g in 1 ml. Chouch and his group of workers (2) used *Lactobacillus Leichman-nii* in determinations of vitamin B<sub>12</sub> and they reported values of 0.5—1.0 m $\mu$ g per ml of whole blood.

Since the reported values are so divergent, the writer attempted to check them by employing the method described by Burkholder in 1951, in which the test organism is a mutation of *E. coli*, strain No. 113—3. This method is suitable for determinations of vitamin B<sub>12</sub> from blood plasma if the growthpromoting action on *E. coli* of free methionine in the blood plasma can be eliminated. This can be effected by making aqueous dilutions of the blood plasma in the proportions of 1: 50 and 1: 100. When these dilutions are used, the final filtrates to be tested will contain such a small amount of methionine that it is incapable of stimulating the growth of the bacterium. In a part of the plasma samples the writer made determinations of the methionine content according to Davis and Mignoli (3) and obtained average values of 0.7—1.0 mg per 100 ml. According to Burkholder, the ratio between the amounts of vitamin B<sub>12</sub> and

methionine capable of producing an equally marked growth of *E. coli* strain 113—3 is 1:50,000. The amounts of methionine found to be present in plasma are nevertheless so small that the dilutions mentioned are sufficient to eliminate their effect.

The detailed procedure in these tests was the following. To ml of citrated plasma obtained by centrifugation were diluted with 8 ml of water. The pH was adjusted to 5.1 with 1-n HCl and the solution was heated in a boiling water-bath for 40 min. During this period the proteins in the solution were completely precipitated and the vitamin B<sub>12</sub> determination was made from the clear supernatant fluid.

The writer of the present report also added to the plasma samples varying amounts of the aqueous solution of B<sub>12</sub> and carried out recovery tests. It was possible to recover 75—90 per cent of the added substance.

Determinations were made of the vitamin B<sub>12</sub> level of the blood plasma of 5 healthy persons and of 2 patients with pernicious anaemia. The results are given below.

Healthy Subjects	Vitamin B <sub>12</sub> in 1 ml of Blood Plasma
No. 1	22 mμg
» 2	25 »
» 3	12 »
» 4	10 »
» 5	10 »
Patients with Pernicious Anaemia	
No. 1	4 »
» 2	6 »

The complete series of tests is still at its initial stage. To check the results it will be necessary to make the determinations by several different methods. The findings will be reported later.

#### REFERENCES

1. BURKHOLDER, P.: *Science* 1951:144:459.
2. CHOUGH, F.: *Amer. J. Physiology* 1950:77:163.
3. DAVIS, B., and MIGNOLI, E.: *J. Bact.* 1950:60:17.
4. ROSS, G. I. M.: *Nature* 1950:166:270.
5. UNGLEY, C. C.: *Nutrition Abst. and Rev.* 1951—1952:21:1—26.

*Addendum.* — After correspondence with Mr. Ross, a corrigendum to this paper has been found necessary. The unit μμgm used by him in his work is not equal to mμgm but means 1/1000 mμgm. Furthermore, there exists no greater discrepancies between the values reported by Ross and those of Chouch and his co-workers.

## DISTRIBUTION OF LIPIDS IN SERUM PROTEIN FRACTIONS SEPARATED BY ELECTROPHORESIS IN FILTER PAPER

by

ESKO NIKKILÄ

(Received for publication August 21, 1952)

Paper electrophoresis offers better facilities for the isolation of pure protein fractions than the classical Tiselius procedure. Thus, the exact composition of the electrophoretically separable protein components of e.g. blood serum in health and disease can be determined. An investigation on the lipid content of paper-electrophoretically separated serum protein fractions was undertaken as a part of a more extensive study on serum lipoprotein metabolism. This paper presents certain preliminary data on the subject; more detailed results will be published shortly.

### METHOD

Electrophoresis was performed using Munktell 20 filter paper and an apparatus constructed by the writer in which 0.25 ml of serum could be fractionated in 4 hours. This amount suffices for an exact analysis of cholesterol or phospholipid from each fraction. The strips were dried at room temperature and the albumin fraction localized by means of its *fluorescence in ultraviolet light*. Then a narrow piece of paper was stained with bromphenol blue to localise the other protein fractions and the strips cut at given distances from the fluorescence zone. Control stainings showed the accuracy of this method. Each separated fraction was extracted with ethanol-ether (3: 1) in slightly boiling water bath and the filtered extracts analysed for cholesterol and lipid phosphorus by the method of

Vesa and Kalaja using Beckman spectrophotometer. The values were corrected for the small lipid content of the filter paper. The recovery was good.

#### RESULTS

The results of analyses made from 10 normal and 2 atherosclerotic human sera and one hypercholesterolemic rabbit serum are presented in Table 1. The results show that with this method all protein fractions are found to contain both cholesterol and phospholipid,  $\beta$  globulin being richest in lipid material. A surprisingly high  $\gamma$  globulin lipid value is found. An interesting change in the distribution of lipids is noted in all sera received from individuals with active atherogenesis. This includes a shift of lipids from  $\alpha$  globulins to slower fractions.

#### DISCUSSION

According to Blix, Tiselius and Svensson (2) all protein fractions of human serum separated in free electrophoresis contain lipids,  $\gamma$  globulin however, only traces. In paper electrophoresis Kunkel and Tiselius (8) have suggested the existence of 3 separated cholesterol maxima. Recently Swahn (15), by staining the serum lipids after paper electrophoresis with Sudan black, has distinguished 4 components, the slowest of which remains close to the starting point. In low-temperature ethanol fractionation, on the other hand, the plasma lipids are almost exclusively found in the form of two well-defined lipoproteins, called according to their electrophoretic mobilities  $\alpha_1$  and  $\beta_1$  lipoproteins (9, 14). The relatively high lipid content of  $\gamma$  globulin obtained in paper electrophoresis is in contradiction to earlier experience. It seems likely that this is only apparent and due to some technical reason. First,  $\beta$  lipoprotein (or a part of it, probably *i.a.* the lipoprotein molecules described by Gofman *et al.* [3], having a relatively large particle size, can be readily absorbed in filter paper thus leaving a trail to the position of  $\gamma$  globulin. Second, a part of the serum lipids is probably unassociated with proteins (or associated with only traces of protein) and does not migrate at all (or migrates very slowly) in an electrical field. Swahn (15) supposes that the lipid material near the starting point is composed of chylomicrons. This cannot, however, be the only explanation since chylomicrons have a very low cholesterol content.

TABLE 1  
PERCENTAGE DISTRIBUTION OF CHOLESTEROL (Ch) AND PHOSPHOLIPID (Pl) BETWEEN SERUM PROTEIN FRACTIONS SEPARATED BY PAPER ELECTROPHORESIS

Subject	Albumin		$\alpha_1$ -glob.		$\alpha_2$ -glob.		$\beta$ -glob.		$\gamma$ -glob.		$\beta + \gamma$ -glob.	
	Ch	Pl	Ch	Pl	Ch	Pl	Ch	Pl	Ch	Pl	Ch	Pl
10 normal males age 19-27 years, range	2.5-6.0	6.0-13.0	17.0-25.5	33.5-44.0	9.0-12.0	13.5-17.0	37.0-52.5	25.5-37.0	14.5-23.5	10.5-18.5	61.0-70.0	42.0-53.0
Myocardial infarction male 46	4.5		13.5		5.0		53.0		24.0		77.0	
Myocardial infarction male 53	3.5		12.0		8.0		51.5		26.0		77.5	
Hypercholesterolemic rabbit	2.5		8.0		8.0		54.0		27.5		81.5	

In free electrophoresis chylomicrons are observed to migrate with  $\alpha$  or  $\beta$  globulins or between them (10). Third, an interaction of  $\beta$  lipoprotein with  $\gamma$  globulin (6) must be taken into consideration, although this seems less probable under these experimental conditions (pH 8.6, ionic strength 0.05). In the opinion of the present author the absorption theory seems most likely. It is further supported by the about equal cholesterol/phospholipid ratio found in  $\beta$  and  $\gamma$  globulins. For this reason the lipid values of combined  $\beta$  and  $\gamma$  globulins are presented and thought to represent the true amount of lipid bound to  $\beta$  lipoprotein.

The extent to which the lipids found in the  $\alpha_2$  globulin region belong to  $\alpha_1$  lipoprotein absorbed in the paper or to a separate  $\alpha_2$  lipoprotein is a problem that remains to be solved. The existence of this lipoprotein has been suggested previously by the ultracentrifugal studies of Green and Lewis (5).

The albumin fraction of all sera examined contained more or less lipid material. This can be due to the existence of a special lipoprotein entity having this mobility. However, it is quite possible that the  $\alpha_1$  lipoprotein does not possess exactly the same mobility as  $\alpha_1$  globulin, but a little greater. In this connection attention should be devoted to the fact that the classification of serum lipoproteins to  $\alpha_1$  and  $\beta_1$  moieties is based on the mobility measurements made from fractions which have been separated by chemical means. Bearing the easy denaturability of just these complexes in mind, it is questionable whether the electrophoretic mobilities remain completely unchanged during the isolation process. In view of the supposed structure of lipoproteins it is further probable that the mobility of a given lipoprotein is altered by the variation of its lipid content (and lipid composition). This enters into question especially in various pathological states, which produce quantitative and/or qualitative changes in the serum lipids. Further studies are needed to solve this problem.

Supposing average normal values for the protein fractions in the sera investigated by Blix, Tiselius and Svensson (2), the approximate percentage distribution of lipids can be calculated from their data. Thus, from cholesterol 64 per cent is found in  $\beta$  globulin, and 35 per cent in faster-migrating fractions (their  $\alpha_1$  globulin is included in albumin), the corresponding percentages for phospholipids being 42 and 45, respectively. Although these values can be

criticized on the basis of the smallness of the material and of the apparent technical difficulty of obtaining absolutely pure fractions, they are in good accordance with the present data. So also are the cholesterol/phospholipid ratios of each fraction.

The most extensive studies on protein-lipid relationships in human plasma reported thus far have been made by Russ, Eder and Barr (14), using low-temperature ethanol fractionation (method 10 of Cohn). Almost all lipids are found in two fractions (A and C), corresponding to the  $\alpha_1$  and  $\beta_1$  lipoproteins. Although the lipid content of each fraction is much more variable than in the present material the means coincide well. In making comparisons, however, it must be noted that the present series includes only male subjects from rather narrow age limits, and that the effect of both age and sex on serum lipoproteins has been suggested by several authors (7, 14). Investigations with the present method into the correlation of lipid distribution with age and sex are in progress.

Although the pathological material examined by the electrophoretic technique is as yet too small to permit definite conclusions, it shows that alterations in the lipid distribution occur in pathological states which are known to be related in some way to disturbances in serum lipids. Of these atherosclerosis is of special interest because of the presumed role of a lipoprotein abnormality in its pathogenesis (3, 1). The observations made by the present method in atherosclerotic sera indicate a decrease in  $\alpha$  lipoprotein cholesterol and a corresponding absolute and relative increase in the cholesterol bound to slower fractions (especially to that found in the position of  $\gamma$  globulin) as compared with normal values. They thus support the results obtained recently by ethanol fractionation (1), and by ultracentrifugal flotation (13) techniques. It is believed that these findings probably also explain the appearance of those lipoprotein particles which Gofman and his associates (3, 4) have correlated with the occurrence of atherosclerosis ( $S_r$  12—20 and other lipoprotein classes). In speculating on the possible causes of this abnormality the findings regarding the role of heparin are of particular interest. It has been shown that a decrease of the heparinoid substances of blood occurs in atherosclerosis (or as a result of aging) (12). On the other hand electrophoretic experiments have suggested that heparin causes a shift of lipids from  $\beta$  to  $\alpha$  globulins (11).

## SUMMARY

Preliminary results of the cholesterol and phospholipid analyses made from serum protein fractions separated by means of paper electrophoresis are presented. All fractions were found to contain both lipids, the greatest amount in  $\beta$  globulin. The results are discussed in connection with the earlier experience of serum lipid-protein relationships. Investigations made from sera of atherosclerotic subjects suggested, in accordance with earlier results obtained by other methods, a shift of lipids from  $\alpha$  to  $\beta$  globulins. This finding is discussed briefly.

## REFERENCES

1. BARR, D. P., RUSS, E. M., and EDER, H. A.: *Am. J. Med.* 1951:11:480.
  2. BLIX, G., TISELIUS, A., and SVENSSON, H.: *J. Biol. Chem.* 1941:137:485.
  3. GOFMAN, J. W., LINDGREN, F., ELLIOTT, H., MANTZ, W., HEWITT, J., STRISOWER, B., HERRING, V., and LYON, T. P.: *Science* 1950: 111:166.
  4. GOFMAN, J. W., JONES, H. B., LYON, T. P., LINDGREN, F., STRISOWER, B., COLMAN, D., and HERRING, V.: *Circulation* 1952:5:119.
  5. GREEN, A. A., and LEWIS, L. A.: 119 th Meeting of American Chemical Soc., Div. Biol. Chem. April 1951. Boston. — Cited by RUSS, EDER, and BARR (14).
  6. GURD, F. R. N., ONCLEY, J. L., EDSALL, J. T., and COHN, E. J.: *Faraday Soc. Disc.* 1949:6:70.
  7. JONES, H. B., GOFMAN, J. W., LINDGREN, F. T., LYON, T. P., GRAHAM, D. M., STRISOWER, B., and NICHOLS, A. V.: *Am. J. Med.* 1951:11:358.
  8. KUNKEL, H. G., and TISELIUS, A.: *J. Gen. Physiol.* 1951:35:89.
  9. LEVER, W. F., GURD, F. R. N., UROMA, E., BROWN, R. K., BARNES, B. A., SCHMID, K., and SCHULTZ, E. L.: *J. Clin. Investig.* 1951: 30:99.
  10. McFARLANE, A. S.: *Faraday Soc. Disc.* 1949:6:74.
  11. NIKKILÄ, E. A.: *Scand. J. Clin. & Lab. Investig.* 1952. (in press).
  12. NIKKILÄ, E. A., and MAJANEN, S.: *ibid.* 1952. (in press).
  13. PRATT, H. M.: *Federation Proc.* 1952:11:270.
  14. RUSS, E. M., EDER, H. A., and BARR, D. P.: *Am. J. Med.* 1951:11:468.
  15. SWAHN, B.: *Scand. J. Clin. & Lab. Investig.* 1952:4:98.
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## CONTINUOUS INTRAVENOUS ADRENALIN AND NORADRENALIN INFUSION

EFFECT ON CIRCULATION AND CERTAIN BLOOD CARBOHYDRATES,  
LIPIDS, AND PHOSPHORUS FRACTIONS IN NORMAL PERSONS AND  
PATIENTS WITH NEUROCIRCULATORY ASTHENIA

by

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(Received for publication August 22, 1952)

### INTRODUCTION

Recent studies have shown that noradrenalin liberated from the sympathetic nerve endings, as well as adrenalin, is a potent regulator of the circulation (2, 8, 9). In view of the different mode of action of these two sympathetic neurohormones as regulators of the circulation and of the blood chemistry, we deemed it advisable to study their importance in neurocirculatory asthenia (NCA) as compared with normal persons. The diseases associated with the autonomic nervous system have been fairly little studied. As is known, disturbances referable to the autonomic nervous system are often observed in this syndrome, at least in the circulation, but to some extent also in the blood chemistry. On the other hand, we know that the functions controlled by the autonomic nervous system are greatly influenced for instance by endocrine factors. The extirpation of the adrenal, thyroid, or pituitary glands reduces the effect of adrenalin in the regulation of the blood pressure and of blood sugar, while for instance thyroxin, adrenal cortical hormones, and pituitary hormones increase these effects.

The impulses of the parasympathetic system may also contribute since they are antagonistic to the functions of the sympathetic

system. The ganglion-blocking substances may cause orthostatic collapse — even in clinical doses. The medical therapy in NCA also indicates the importance of autonomic and endocrine functions in this syndrome.

The effect of continuous adrenalin infusions of long duration on the circulation and on certain chemical substances in the blood has been previously studied by Pekkarinen & Hortling 1951 (10). In a preliminary study concerning the various blood phosphorus fractions in patients with NCA as compared with normal controls (6) we found no typical differences. Helve (3, 4, 5) has also studied the distribution of phosphorus fractions of blood in certain pathological conditions, e.g. in Addison's disease after adrenalin infusion.

In the present study we are dealing with the effect elicited by adrenalin and by noradrenalin, each administered as one continuous intravenous infusion, on the circulation and on certain substances of the carbohydrate, phosphorus, and lipid groups in patients with NCA and normal controls of equal age.

#### MATERIAL AND METHODS

A series of servicemen was studied who were treated at the Central Military Hospital 1 for NCA, diagnosis being based on the criteria described below. Great importance was attached to the symptoms for which they had been referred to the Hospital from their units. These symptoms appear in detail in Table 1. Before reaching the Central Military Hospital 1 each patient had been examined by the Army Medical Officers of his respective units. Other diseases chiefly thyrotoxicosis, myocarditis, and ordinary neurotic conditions were as far as possible excluded at the clinical examination; the same applies to cases of simulations. The ages of the patients were from 20 to 22 years, and the body weight from 57 to 78 kg. When the patients had to stand for at least 3 minutes, the systolic pressure fell at least 5 mm Hg, and the pulse rate increased by at least 20 beats per minute in a half of the patients. In several cases roentgenologic examination showed a »droplet-shaped» heart. Electrocardiographic examination at rest and after exertion revealed no pathologic changes apart from tachycardia, but (Lead II) in the erect posture showed in some of the cases a distinct fall of the T wave: in 4 cases it was isoelectric, in 3 cases depressed, in the rest there were no electrocardiographic abnormalities. The patients all had normal blood pictures. The temperature recorded in the axilla often showed instability, and occasionally a tendency to hyperthermia, though infections had been ruled out by observation of the sedimentation rate, leukocyte count and by other investigations. We have reached the opinion that patients with NCA have a tendency to slight elevation of temperature.

TABLE I  
SIGNS AND SYMPTOMS OF NCA-PATIENTS

Age years	Length cm	Weight kg	Cor (rtg)	Ekg	Blood Pressure mm Hg		Heart Rate/Min.		Tired- ness	Ver- tigo	Nervous- ness	Compres- sion in the Cardiac Region	Palpi- tation	Moist Hands and Feet	Tempe- rature
					Lying	Standin	Lying	Standin							
21	186	75	0	+	130/65	155/70	68	76	+	±	+	+	+	±	+
22		75	0	+	120/75	130/80	72	96		±	±	+	+	+	—
21	171	57	0	—	140/85	130/85	100	104	+	+	+	+	+	±	+
20	178	70	0	±	130/70	140/85	56	84	+	+	+	+	+	±	+
21	136	68	0	±	130/75	125/70	68	98	+	+	+	+	+	±	+
20	180	78	0	—	140/80	130/75	64	66	±	+	±	+	+	±	—
31	172	70	0	—	120/70	115/80	76	104	±	±	±	+	+	±	—
20		65	0	—	115/80	110/75	68	108	±	+	+	+	+	+	+
22	169	59	0	±	125/80	110/75	72	84	+	+	+	+	+	+	—
21	159	64	0	+	150/90	140/90	72	80	±	—	—	+	+	±	—
22	172	72	0	—	140/85	135/70	72	80	±	+	+	+	+	±	—
20	190	69	0	—	140/85	115/85	60	96	+	±	±	+	+	±	+
20		59	0	±	125/75	120/80	80	96	+	±	±	+	+	±	+
20			0	—	120/70	120/70	68	84	+	±	±	+	+	±	+
20		65	0	+	140/90	115/85	64	96	+	+	+	+	+	+	+

+ — = decrease of T<sub>II</sub>  
Ekg: + = isoelectric T<sub>II</sub>  
+ + = negative T<sub>II</sub>

Temperature: + = ad 37.2—37.3° C

The infusion was administered by the Kurode-Straub apparatus, with which the rate of a continuous infusion can be regulated. 1.2 ml of »Exadrin» Astra (1 in 1,000) in 7 ml of normal saline was injected into the cubital vein of 27 servicemen, 15 belonging to the group of NCA and 12 normal controls. Using the same method of administration, 1.2 ml of »Norexadrin» Astra (1 in 1,000) was injected into 10 subjects, 5 with NCA and 5 normal controls. The infusion rate in each group averaged 0.2 yg/kg/min. and the duration of infusion 40 to 45 minutes. The total dose of adrenalin and of noradrenalin varied from 0.6 to 0.7 mg, which equals about 10 per cent of the total amount of catechols in the adrenal glands.

The tests were made in the morning after an overnight fast and the patients were kept in bed throughout the test. The patients were observed for any subjective symptoms, the systolic and the diastolic pressure were recorded during the infusion and 1½ hours after it, and the following determinations made in the whole blood: sugar, inorganic phosphate, pyrophosphate-P, hexosephosphate-P, diphosphoglyceric acid-P, total acid-soluble P, total phosphorus, lactic acid; in the plasma: inorganic phosphate, total P, total lipin phosphorus; in the serum: ester cholesterol, total cholesterol, and potassium. Samples were withdrawn from the cubital vein before the infusion, ½ hour after it was started, and 1½ hours after it was stopped; blood sugar, however, was determined from the blood of the finger tip. The following methods were used: Hagedorn-Jensen's for blood sugar, Bomskov's method modified by Helve for phosphorus fractions, Liebermann-Burchard's modified by Kalaja (7) for serum cholesterol, Winnich's (1) for lactic acid, and Kramer's sodium-cobalt nitrite method for potassium.

The results in the three time groups (before, during, and after the test) have been statistically studied, comparing the changes in the normal group with those observed in the patients with NCA, and also the changes produced by adrenalin with those produced by noradrenalin in the patients and in the controls.

The normal controls, of equal age as the patients with NCA, were convalescents under treatment for some unassociated mild complaint. Their blood pictures and sedimentation rates were normal. They had no subjective symptoms, nor did they show any signs of disease. Their chest and heart roentgenograms showed no pathological changes.

#### CIRCULATORY CHANGES

(Tables 2, 3, 4, Fig. 1)

1. *Systolic Blood Pressure.* — Before the test the systolic pressure was much the same in the groups studied, 135–138 mm. Hg. (Table 2).

*Adrenalin* infusion increased the systolic pressure in the group of NCA by 30 mm Hg (+ 21.4 per cent); and in the control group

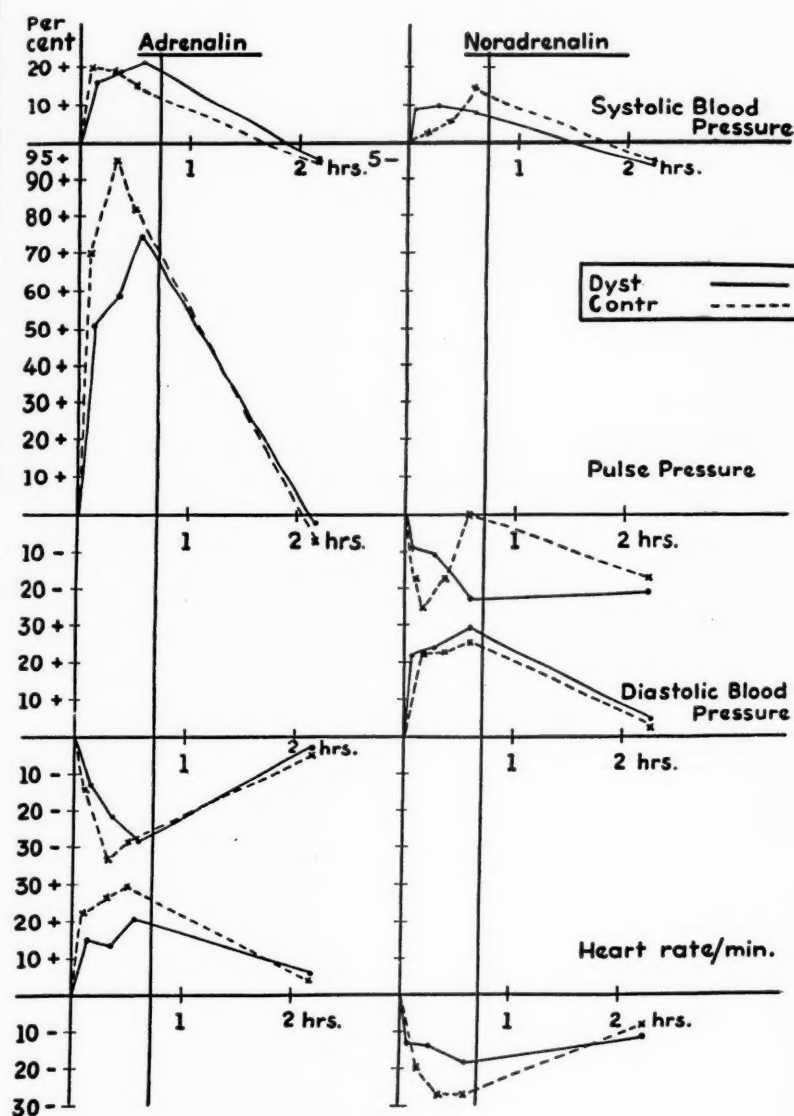


Fig. 1.

The effects of continuous intravenous adrenalin and noradrenalin infusions on the circulation in man in the control- and NCA-groups. The vertical line indicates the cessation of the infusion.

I = NCA (Neurocirculatory asthenia).  
II = Controls.

TABLE 2  
THE EFFECTS OF CONTINUOUS INTRAVENOUS ADRENALIN

		Adrenalin									
		The mean before	Mean Changes during Infusion								
			T <sub>1</sub> I = 9 min II = 7 min			T <sub>2</sub> I = 21 min II = 19 min			T <sub>3</sub> I = 34 min II = 32 min		
			x	t	n	x	e	n	x	t	n
Systolic Blood Pressure, I	138	+22.0	3.62**	(13)		+26	5.01***	(12)	+30	6.40***	(14)
mm Hg	II	138	+28	8.86***	(12)	+26	14.1 ***	(12)	+22	5.32***	(12)
Diastolic Blood Pressure I	82	-11	1.88	(12)		18	2.79*	(12)	-23	2.82*	(14)
mm Hg	II	83	-12	2.62*	(12)	-28	3.42**	(12)	-24	3.40**	(12)
Pulse Pressure, I	60	+31	3.55**	(12)		+35	4.49***	(12)	+45	4.35**	(14)
mm Hg	II	56	+39	6.21***	(12)	+53	6.37**	(12)	+46	4.56***	(12)
Heart Rate/min	I	68	+10.1	4.24***	(13)	+ 9.3	3.72****	(12)	+14	3.78**	(14)
	II	75	+17	4.00**	(12)	+20	4.68**	(12)	+22	4.85***	(12)

x = the mean changes compared with the mean before the infusions. t = probability (\* 95 \*\* 99 \*\*\* 99.9)

TABLE  
COMPARISON OF THE CIRCULATORY CHANGES BETWEEN THE CONTINUOUS CONTROL

	Controls							
	During Infusion				1 ½ hrs. after Infusion			
	T <sub>1</sub> (8 min)		T <sub>2</sub> (20 min)			T <sub>3</sub> (35 min)		
	x	t	x	t		x	t	
Systolic Blood Pressure, mm Hg .....	+24	3.67**	+18	2.62*	+2	0.25	0	0.02
Diastolic Blood Pressure, mm Hg .....	-29	3.41**	-46	3.36**	-44	3.49**	-6	1.37
Pulse Pressure, mm Hg	+53	4.70***	+63	4.59***	+46	2.55*	+5	0.81
Heart Rate/min. ....	-31	4.46***	-40	5.86***	+42	5.21***	+9	1.51

x = the mean difference of changes between adrenalin and noradrenalin

TABLE  
COMPARISON OF THE CIRCULATORY CHANGES BETWEEN NCA- AND CONTROL ADRENALIN

	Adrenalin							
	During Infusion				1 ½ hrs. after Infusion			
	T <sub>1</sub> (8 min)		T <sub>2</sub> (20 min)			T <sub>3</sub> (35 min)		
	x	t	x	t	x	t	x	t
Systolic Blood Pressure, mg Hg .....	—6	0.74	0	0	+8	1.21	+2	0.57
Diastolic Blood Pressure, mm Hg .....	+1	0.13	+ 9	0.88	+1	0.08	+1	0.05
Pulse Pressure, mm Hg	—8	0.73	—18	1.62	+1	0.05	+3	0.52
Heart Rate/min. ....	—7	1.46	—11	2.20*	—8	1.26	+1	0.15

x = the mean difference of changes between NCA- and control groups.

TAB. 2  
INTRA-VENOUS ADRENALIN AND NORADRENALIN INFUSIONS ON THE CIRCULATION

		Noradrenalin													
1 ½ hrs. after Infusion		Mean before	Mean Changes during Infusion									1 ½ hrs. after Infusion			
			T <sub>1</sub> I = 6 min II = 10 min			T <sub>2</sub> I = 16 min II = 22 min			T <sub>3</sub> I = 36 min II = 35 min						
x	t		x	t	n	x	t	n	x	t	n	n	t		
* (14)	-5	2.08	138	+13	1.44	(5)	+14	1.49	(5)	+11	1.10	(5)	- 8	2.36	
* (12)	-7	2.54*	135	+ 4	0.60	(5)	+ 8	0.80	(4)	+20	2.83	(5)	- 7	2.06	
(14)	-3	1.40	81	+18	1.94	(5)	+20	2.20	(5)	+24	2.59	(5)	+ 4	0.78	
(12)	-4	1.62	78	+18	2.94	(5)	+18	2.40	(4)	+20	5.66*	(5)	+ 2	0.59	
(14)	-1	0.42	57	- 5	1.41	(5)	- 6	1.04	(5)	-13	3.20*	(5)	-12	4.71**	
(12)	-4	1.01	57	-14	5.75*	(5)	-10	1.53	(4)	0	0	(5)	- 9	1.96	
(14)	+4	1.50	71	- 9	1.93	(5)	- 9	2.05	(5)	-13	3.12*	(5)	- 7	3.63*	
(12)	+3	0.85	75	-14	4.20*	(5)	-20	11.2 ***	(4)	-20	9.01**	(5)	- 6	4.24*	

\* 95 %, \*\* 99 %, \*\*\* 99.9 %). n = number of cases.

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#### INTRA-VENOUS ADRENALIN AND NORADRENALIN INFUSIONS IN THE NCA- AND GROUPS

NCA							
During Infusion						1 ½ hrs. after Infusion	
T <sub>1</sub> (8 min)		T <sub>2</sub> (18 min)		T <sub>3</sub> (35 min)			
x	t	x	t	x	t	x	t
+ 9.0	0.76	+12	1.16	+19	1.94	+ 3	0.66
—29	2.60*	—38	3.24**	—47	3.33**	— 7	1.49
+36	2.32*	+41	3.20**	+58	3.52**	+11	1.98
+19	3.94**	+19	3.84**	+27	4.21***	+11	2.46*

infusions. t = probability (\*95 %, \*\* 99 %, \*\*\* 99.9 %).

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#### GROUPS DURING THE CONTINUOUS INTRA-VENOUS ADRENALIN AND NOR- INFUSIONS

Noradrenalin							
During Infusion						1 ½ hrs. after Infusion	
T <sub>1</sub> (8 min)		T <sub>2</sub> (18 min)		T <sub>3</sub> (35 min)			
x	t	x	t	x	t		
+ 9	0.80	+ 6	0.44	— 9	0.70	—1	0.21
+1	0.04	+ 2	0.17	+ 4	0.36	+2	0.33
+9	2.02	+ 4	0.46	—13	2.15	—3	0.57
+6	0.98	+10	2.00	+7	1.37	—1	0.56

t = probability (\* 95 %, \*\* 99 %, \*\*\* 99.9 %).

by an average of 28 mm Hg (+ 20.0 per cent); these changes are significant. The systolic pressure rose at the very beginning of the infusion in both groups, but it tended to fall slightly in the control group and to rise slightly in the group of NCA towards the end of the infusion. Within  $1\frac{1}{2}$  hours after the infusion was stopped the blood pressure was somewhat lower in both groups (17 mm Hg) than before the test.

*Noradrenalin* caused the systolic pressure to rise less (by 14–20 mm Hg) than did *adrenalin*. The changes were slight. After termination of the infusion the systolic pressure was lower than before the test (by 7–8 mm Hg).

Comparison of the effects of *adrenalin* and *noradrenalin* (Table 3) shows that the former increased the systolic pressure of the controls more than *noradrenalin* in the first time group (statistically significant difference).

There is no typical statistical difference between the changes in the normal controls and in the patients with NCA (Table 4).

2. *Diastolic Blood Pressure.* — Before the test the diastolic pressure was much the same (78–83 mm Hg) in the test group (Table 2).

*Adrenalin* caused a fall in the diastolic pressure during the infusion. Immediately on beginning of the infusion the fall in diastolic pressure was 11 mm Hg, and later 28 mm Hg or — 33 per cent in the control group and 23 mm Hg or — 28 per cent in that of NCA which changes are significant.  $1\frac{1}{4}$  hours after the infusion the diastolic blood pressure was fairly normal (3–4 mm Hg).

*Noradrenalin*, in contrast to *adrenalin*, increased the diastolic pressure. At the very beginning of the infusion this pressure rose by 18 mm Hg (+ 22 per cent) in both groups and at the end of the infusion the rise was 24 mm Hg (+ 30 per cent) in the group of NCA, and 20 mm Hg (+ 26 per cent) in the control group.  $1\frac{1}{2}$  hours after the end of the infusion the diastolic pressure was still slightly elevated.

When *adrenalin* lowers the diastolic pressure and *noradrenalin* increases it, there is a highly significant statistical difference in the response to these substances (Table 3). The difference between the respective changes in both groups was 47 mm Hg in the group of NCA and 46 mm Hg in the control group. In this respect the response to *adrenalin* differs strikingly from that to *noradrenalin*.

There is no statistical difference between the control group and that of NCA during adrenalin or noradrenalin infusion (Table 4).

3. *Pulse Pressure.*—Before the infusion the pulse pressure were in the group of NCA 57–60 mm Hg and in the controls 56–57 mm Hg (Table 2).

*Adrenalin*, which increases the systolic pressure and reduced the diastolic pressure, caused the pulse pressure to rise very considerably during the infusion. In the control group the pulse pressure rose somewhat more (by 53 mm Hg, or +95 per cent) than in that of NCA (by 45 mm Hg, or +75 per cent). The increase in pulse pressure is highly significant in both groups.

*Noradrenalin* increased both the systolic and the diastolic pressure to some extent, and often the latter slightly more; because of this the pulse pressure did not rise: it was somewhat lower (by 13–14 mm Hg, or 23–24 per cent) after the infusion than before it.

Comparison of the effects of adrenalin and noradrenalin shows that the difference between the pulse pressure is statistically significant (Table 3). However, there is no typical difference between the group of patients with NCA and the control group (Table 4).

4. *Heart Rate.*—Before the infusion the heart rate was 68–71/min. in the group of NCA and 75/min. in the control group (Table 2).

*Adrenalin* increased the heart rate towards the end of the infusion significantly, 14.3/min. or +21 per cent in the group of NCA, and 22/min. or +29 per cent in the control group. This acceleration was greater in the controls than in the patients with NCA.  $1\frac{1}{2}$  hours after the infusion was stopped, the pulse rate had returned almost to normal, yet it tended to be slightly (3–4 beats) more rapid.

*Noradrenalin* produced a rather distinct fall in the heart rate. In the control group fall was 20/min. or –27 per cent and in that of NCA 13/min. or –18 per cent. This change was also more distinct in the control group than in that of NCA.  $1\frac{1}{2}$  hours after termination of the infusion the pulse rate tends to be slightly (6–7 beats) below the normal.

Comparison of the effects of adrenalin and noradrenalin shows that they differ greatly: in the control group adrenalin causes acceleration of the heart rate and noradrenalin causes retardation

(difference 42/min.) (Table 3). In the group of NCA the difference between the heart rates produced by adrenalin and by noradrenalin is not so great (27/min.) These changes are highly significant. The pulse rate response of these patients to adrenalin and to noradrenalin seems to be less than in the control group (Table 4). Slight changes in the pulse rate seem to be characteristic in NCA. It is evident that these patients show failure of regulation in this respect.

The difference in pulse rate response between the controls and NCA is obviously significant (Table 4).

#### CHANGES IN CARBOHYDRATE, PHOSPHORUS AND LIPOID METABOLISM OF THE BLOOD

(Tables 5, 6, 7, Fig. 2)

1. *Blood Sugar.* — Before the infusion blood sugar was 84–89 mm Hg in the group of NCA and 83–85 mm Hg in the controls (Table 5).

*Adrenalin* raised the blood sugar in the NCA-group by 60 mg. per hundred 1 ml (+ 71 per cent) and in the control group by 80 mg per hundred 3 ml (+ 97 per cent). These changes are highly significant statistically.  $1\frac{1}{2}$  hours after the infusion was stopped the blood sugar was practically normal.

*Noradrenalin* infusion increased the blood sugar level fairly little; in the control group by 22 mg per hundred ml (+ 26 per cent), and in the NCA group still less, by 11 mg per hundred ml (+ 12 per cent) (Table 5). After termination of the infusion the blood sugar was relatively normal.

Comparison of the effects of adrenalin and noradrenalin shows that the former increases the blood sugar greatly, the latter but little. The difference between these changes is also highly significant: in the control group 49 mg per hundred ml, in that of NCA 58 mg per hundred ml (Table 6).

In the patients with NCA the rise in blood sugar level was less than in the control group. The difference between the changes produced in the two groups during the adrenalin infusion is significant (21 mg per hundred ml) (Table 7).

Though the rise was less in NCA-group than in the controls during noradrenalin infusion, the difference between the responses is not statistically significant (Table 7).

**TABLE 5**  
THE EFFECTS OF CONTINUOUS INTRAVENOUS INFUSIONS OF ADRENALIN AND NORADRENALIN ON THE BLOOD CHEMISTRY IN MAN  
I = NCA (Neurocirculatory asthenia). II = Controls

TABLE 6

COMPARISON OF THE CHANGES IN THE BLOOD CHEMISTRY BETWEEN THE CONTINUOUS INTRAVENOUS ADRENALINE AND NORADRENALINE INFUSIONS IN THE CONTROLS AND NCA-GROUPS

	Controls				NCA			
	At the End of Infusion		1 ½ hrs. after Infusion		At the End of Infusion		1 ½ hrs. after Infusion	
	x	t	x	t	x	t	x	t
Blood Sugar mg per cent .....	+58	4.34***	— 4	0.44	+49	4.37***	+2	0.31
Inorganic Phosphate:								
a) Whole blood mg per cent ....	— 0.5	1.29	— 0.2	2.08	— 0.5	1.46	— 0.2	0.83
b) Plasma mg per cent .....	— 0.8	2.92*	— 0.4	1.80	— 0.7	2.09	0	0.05
Pyrophosphate-P mg per cent .....	— 0.1	0.48	+ 0.1	0.74	— 0.1	0.16	— 0.1	0.37
Hexosephosphate-P mg per cent ....	+ 0.3	0.92	+ 0.4	0.81	+ 0.4	0.63	+ 0.1	0.10
Diphosphoglyceric Acid-P mg per cent	+ 0.4	0.65	+ 1.0	1.54	— 1.1	0.94	— 0.8	0.62
Total Acid-soluble Phosphorus mg per cent .....	— 1.0	0.78	+ 0.3	0.21	+ 2.1	1.09	— 3.5	1.48
Total Phosphorus:								
a) Whole blood mg per cent .....	— 2.4	1.47	— 0.9	0.58	— 2.5	1.21	— 3.5	1.43
b) Plasma mg per cent .....	— 1.0	1.08	+ 0.2	0.55	— 2.1	1.67	— 1.6	1.20
Total Plasma Lipin P mg per cent .....	+ 0.2	0.19	+ 0.7	0.48	— 0.8	1.53	— 1.3	2.31*
Total Serum Cholesterol mg per cent ..	— 36	2.02	— 10	0.61	— 3	0.45	— 7	0.45
Ester Cholesterol mg per cent .....	— 4	0.35	+ 2	0.25	+ 8	0.89	+ 5	0.50
Lactic Acid mg per cent .....	+ 16	4.19***	+ 6	1.54	+ 8	1.96	+ 3	0.86
Serum Potassium mg per cent .....	— 2.3	0.77	— 3.5	1.43	— 3.8	1.11	+ 0.7	0.25

x = the mean difference of changes between adrenalin — noradrenalin infusions

t = probability (\* 95 %, \*\* 99 %, \*\*\* 99.9 %).

TABLE 7

COMPARISON OF THE CHANGES IN THE BLOOD CHEMISTRY BETWEEN THE CONTROLS AND NCA-GROUPS DURING CONTINUOUS INTRAVENOUS ADRENALIN AND NORADRENALIN INFUSIONS

	Adrenalin				Noradrenalin			
	At the End of Infusion		1 ½ hrs. after Infusion		At the End of Infusion		1 ½ hrs. after Infusion	
	x	t	x	t	x	t	x	t
Blood Sugar mg per cent .....	-21	2.08*	-6	0.89	-11	1.38	-11	2.05
Inorganic Phosphate:								
a) Whole blood mg per cent.....	-0.1	0.36	-0.1	0.45	-0.1	0.52	-0.1	0.56
b) Plasma mg per cent	+0.4	1.40	+0.4	1.82	+0.2	1.51	0	0.12
Pyrophosphate-P mg per cent .....	-0.3	1.15	-0.3	1.52	-0.4	1.46	-0.1	0.26
Hexosephosphate-P mg per cent .....	+0.1	0.29	-0.5	0.99	+0.1	0.29	0	0
Diphosphoglyceric Acid-P mg per cent	-0.3	0.31	+0.2	0.19	+1.2	3.11**	+2.0	5.39***
Total Acid-soluble-P mg per cent .....	+0.1	0.05	-2.5	1.39	+1.2	2.40*	+1.4	2.26
Total Phosphorus:								
a) Whole blood mg per cent.....	0	0	-1.7	0.77	+0.1	0.08	+0.9	0.85
b) Plasma mg per cent	+0.2	0.16	-0.6	0.72	+1.3	1.62	+1.2	1.30
Total Plasma Lipin P mg per cent .....	-0.3	0.55	-0.7	1.07	+0.8	0.50	+1.4	0.78
Total Serum Cholesterol mg per cent ....	-4	0.59	-20	1.61	-37	1.81	-23	1.55
Ester Cholesterol mg per cent .....	+4	0.62	-5	0.79	-8	0.48	-8	0.69
Lactic acid mg per cent	-3	0.98	-3	1.20	+5	1.02	0	0
Serum Potassium mg per cent .....	-0.4	0.15	+3.7	2.04	-1.1	0.32	-0.5	0.12

x = the mean difference of changes between control — and NCA-groups  
t = probability (\* 95 %, \*\* 99 %, \*\*\* 99.9 %).

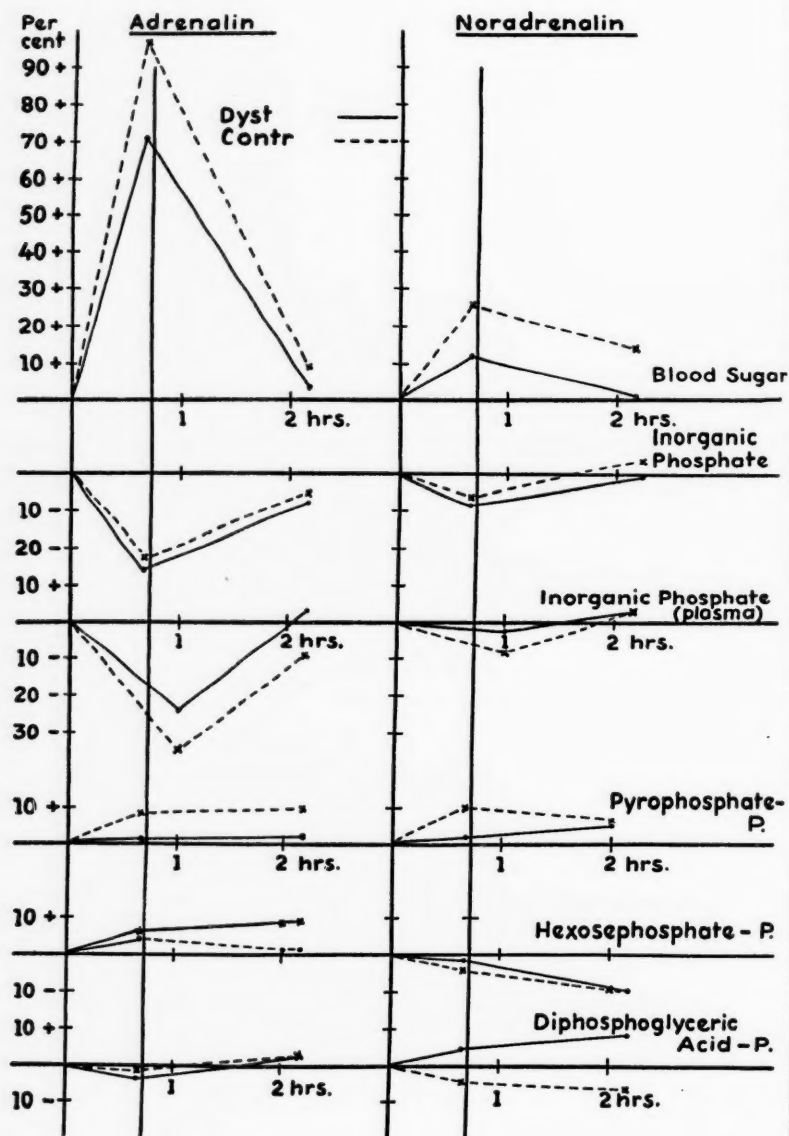
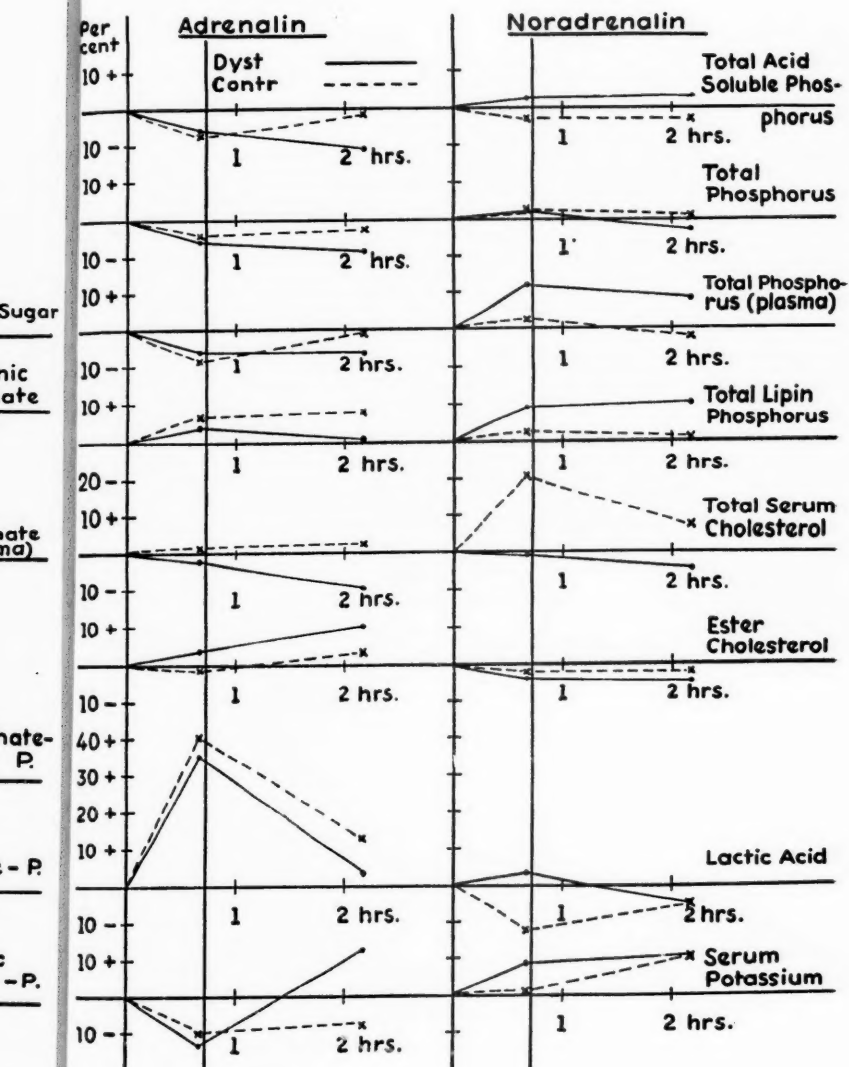


Fig.  
The effects of continuous intravenous adrenalin and noradrenalin  
The vertical line indicates



2. infusions on the blood chemistry in the control- and NCA-groups. the cessation of the infusion.

2. *Inorganic Phosphate: Whole Blood.* — Before the test blood inorganic phosphate was, in the NCA-group 3.1 mg per hundred ml and in the control group 2.8–2.9 mg per hundred ml (Table 5).

*Adrenalin* lowered the blood inorganic phosphate about equally in the control group (by 0.7 mg per hundred ml, or (–23 per cent) and in that of NCA (by 0.8 mg per hundred ml, or (–26 per cent). This drop is significant in both groups.  $1\frac{1}{2}$  hours after cessation of the infusion the content of inorganic phosphate in the blood had returned practically to normal.

*Noradrenalin* produced a weak effect on blood inorganic phosphate, as on blood sugar. The content of inorganic phosphate fell in the group of NCA by 0.3 mg per hundred ml (–10 per cent) and in the control group by only 0.1 mg per hundred ml (–3 per cent). The former change alone is weakly significant statistically (Table 5).

Comparison of the effects showed that the adrenalin lowers the blood inorganic phosphate level relatively much, but the noradrenalin to a slighter extent. The difference between these responses is not significant but probable (Table 6).

There is no statistically significant difference between the changes in the NCA- and control groups during the adrenaline infusion (Table 7).

3. *Inorganic Phosphate: Plasma.* — Before the test the plasma inorganic phosphate was 3.1–3.2 mg per hundred ml in the control group and 3.0–3.2 mg per hundred ml in the group of NCA.

*Adrenalin* decreased the plasma inorganic phosphate during the infusion by 0.7 mg per hundred ml (–24 per cent) in the group of NCA and by 1.1 mg per hundred ml (3) (–35 per cent) in the control group. These changes are statistically significant. After the infusion was stopped the level returned approximately to normal.

*Noradrenalin* reduced the plasma inorganic phosphate content by only 0.2 mg per hundred ml (7 per cent) in the control group, and still less — by 0.1 mg per hundred ml (3 per cent) — in the group of NCA. These changes are not significant. In the response to noradrenalin the two groups show no statistically significant difference.

Comparison of the effects of adrenalin and noradrenalin on plasma inorganic phosphate shows that there is a significant difference in the control group but not in that of NCA (Table 5).

There is no significant difference between the two groups, NCA- and control group during the adrenalin infusion (Table 7).

4. *Pyrophosphate-P*. — Before the infusion the pyrophosphate-P was 2.9—3.4 mg per hundred ml in the group of NCA and 3.7—4.3 mg per hundred ml in the control group (Table 5).

*Adrenalin* infusion increased the pyrophosphate-P by 0.4 mg per hundred ml in the control group after the infusion. This change seems to be significant. There was no corresponding response in the group of NCA. Yet the difference between the two groups is not large enough to be statistically significant (Table 7).

*Noradrenalin* produced a rise of 0.4 mg per hundred ml in pyrophosphate-P during the infusion (table 5). This change is weakly significant statistically.

Comparison of the effects of adrenalin and noradrenalin shows no statistically significant difference between the two groups studied (table 7).

The difference between the NCA- and control groups is not significant, during the adrenalin or noradrenalin infusions (Table 6).

5. *Hexosephosphate-P*. — Before the infusion hexosephosphate-P was in the group of NCA 4.7—3.8 mg per hundred ml, and in the control group its level was 3.9 mg per hundred ml (Table 5).

Infusions of adrenalin and of noradrenalin produced no statistically significant changes in hexosephosphate-P in either of the groups (Table 4), nor did the response to adrenalin differ from that to noradrenalin within the same group (Table 6).

6. *Diphosphoglyceric Acid-P*. — Prior to the infusion the level of diphosphoglyceric acid-P was in the control group of NCA 13.6—14.0 mg per hundred ml and in the control group 13.4—13.5 mg per hundred ml (Table 5).

*Adrenalin* brought about no significant change in either of the groups.

*Noradrenalin* infusion was followed by a rise in diphosphoglyceric acid-P in the group of NCA and by a fall in the control group, the changes being weakly significant. The difference between the two groups seems to be significant (Table 7).

Comparison of the effects of adrenalin and noradrenalin shows no statistically significant difference (Table 6).

7. *Total Acid-soluble P*. — Before the infusion was started the content of total acid-soluble P was in the group of NCA 25.4—23.9

mg per hundred ml and in the control group 24.2—24.3 mg per hundred ml (Table 5).

The infusions of *adrenalin* and of *noradrenalin* were followed by no significant rises or drops in total acid-soluble P in the two groups (Table 5), and in neither of the groups was there a significant difference between the response to adrenalin and that to noradrenalin (Table 6).

8. *Total Phosphorus: Whole Blood.* — In the group of NCA total phosphorus varied from 28.5 to 37.2 mg per hundred ml and in the control group from 32.9 to 32.8 mg per hundred ml before the infusion (Table 5).

9. *Total Phosphorus: Plasma.* — The plasma total phosphorus varied from 10.4 to 12.7 mg per hundred ml in the group of NCA and from 9.5 to 11.0 mg per hundred ml in the control group (Table 5).

Neither *adrenalin* nor *noradrenalin* infusion resulted in significant changes in blood or plasma total phosphorus in the two groups (Table 5).

There is not statistically significant difference between the two groups with respect to the response to adrenalin and to noradrenalin (Table 7).

10. *Total Lipin Phosphorus.* — The average total lipin phosphorus reading before the infusion was 8.5—11.7 mg per hundred ml in the group of NCA, and 8.7—9.7 mg per hundred ml in the control group (Table 5). Neither adrenalin nor noradrenalin produced significant changes in these groups. After the infusion a weakly significant difference can be observed between the effects of adrenalin and of noradrenalin in the group of NCA (Table 6).

11. *Total Serum Cholesterol.* — Before the infusion the total serum cholesterol was 183—209 mg. per hundred ml in the group of NCA, and 185—178 mg per hundred cc in the control group (Table 5).

Neither *adrenalin* nor *noradrenalin* caused any significant changes. A weakly significant rise was observed in the group of NCA after the adrenalin infusion was stopped.

12. *Ester Cholesterol.* — Before the infusion the level of ester cholesterol was 84—136 mg per hundred ml in the group of NCA and 115—130 mg per hundred ml in the control group (Table 5).

Neither adrenalin nor noradrenalin were found to produce any significant changes in the groups studied.

13. *Lactic Acid*. — The blood lactic acid was prior to the infusion 26—32 mg per hundred ml in the group of NCA, and 30—34 mg per hundred ml in the control group (Table 5).

*Adrenalin* raised the value for lactic acid by 9.2 mg per hundred ml (+ 35 per cent) in patients with NCA and by 12.1 mg per hundred ml (+ 40 per cent) in the control group, which changes are highly significant.

*Noradrenalin*, in contrast to adrenalin, did not increase lactic acid: its level was slightly lower after the test than before it. These changes are not significant.

The difference between the changes in the control group and in that of NCA is not statistically significant (Table 7).

14. *Serum Potassium*. — The serum potassium was before the infusion 15.4—17.8 mg per hundred ml in the group of NCA and 19.6—20.1 mg per hundred ml in the control group.

During *adrenalin* infusion the serum potassium fell by 2.0 mg per hundred ml (—10 per cent) in the control group and by 2.4 mg per hundred ml (—13.5 per cent) in that of NCA. These changes are not significant.

*Noradrenalin* infusion caused no significant changes in the serum potassium level.

#### SUBJECTIVE SYMPTOMS

*Adrenalin Infusion* (Table 8). — Adrenalin produced typical symptoms in both the control group and that of NCA. However, certain symptoms were more pronounced in the patients with NCA than in the controls. Tachycardia occurred in both groups, whereas compression in the cardiac region and extrasystoles were observed only in patients with NCA. Restlessness and tremor of the hands were observed in both groups, but tremor of the whole body, vertigo, nausea, headache, scotomata, and visual disturbances only in the group of NCA. It seems that, in patients with NCA, adrenalin infusion often provokes the same symptoms as the patients had complained of when their history was taken.

*Noradrenalin Infusion*. — Noradrenalin generally caused no restlessness, tachycardia or tremor as adrenalin did. During the

TABLE 8  
SYMPTOMS IN NCA- AND CONTROL GROUP DURING THE CONTINUOUS INTRAVENOUS  
INFUSION OF ADRENALIN

	Number of Positive Symptoms	
	NCA (15 Patients)	Control (12 Patients)
Tachycardia .....	8	10
Extrasystoles .....	4	0
Compression in the Cardiac Region ....	5	0
Tremor of the Hands .....	8	10
» » » Body .....	4	0
Restlessness .....	4	3
Vertigo .....	3	0
Headache .....	4	0
Scotomata .....	3	0

infusion some of the patients complained of compression in the cardiac region and mild dyspnoea. These symptoms were more distinct in the NCA group than in the control group.

#### DISCUSSION

A series of servicemen were studied in whom NCA became manifest when they were exposed to unfamiliar Army conditions. Under the ordinary conditions of life they had perhaps been able to avoid sustained or suddenly augmented efforts, but when serving their time in the Army, the circumstances promoted the manifestation of symptoms. Partly these symptoms are the same as those caused by adrenalin infusion.

In an attempt to simulate the mechanism of the sympathetic nervous system we selected a dose of 0.6 to 0.7 mg of adrenalin or noradrenalin given by one continuous infusion lasting 40—45 minutes. This dose is about one-tenth of the normal adrenalin content of the adrenals and therefore it can obviously be regarded as a relatively physiological dose, taking the duration of the infusion into account as well.

The changes produced by adrenalin in the circulation and metabolism differ entirely from those caused by noradrenalin. Adrenalin — as is known — increases the heart rate and the cardiac output, and thus raises the systolic blood pressure, while the diastolic

pressure falls because small physiological doses of adrenalin dilate most of the peripheral blood vessels.

In contrast to adrenalin, noradrenalin contracts most of the peripheral vascular areas and therefore increases both the systolic and the diastolic pressure, but it does not increase heart action. As the peripheral vessels contract, the heartbeat slows down owing to reflex vagal inhibition. Adrenalin increases the heart rate and noradrenalin retards it.

We have here only studied the effect of the sympathetic hormones, without paying particular attention to the parasympathetic nervous system. Yet, when these sympathetic substances are injected, not only the sympathetic tone increases, but reflex changes are also observed in the parasympathetic tone, and these appear very clearly as retardation of the heart rate in connection with noradrenalin infusion. If the vagus nerves were paralysed with atropine, the sympathomimetic effect of noradrenalin would appear as an increase in heart rate.

On the basis of our studies the increase or decrease in heart rate following infusion of adrenalin or noradrenalin, respectively, does not seem to be so great in the patients with NCA as in the controls. It also arouses attention that adrenalin produces similar subjective symptoms in the patients with NCA as they had earlier complained during their military service — compression in the heart region and extrasystoles — both of which the controls did not complain of. Some symptoms referable to the nervous system were also more distinct in the patients with NCA than in the control group: trembling of the whole body, vertigo, nausea, headache, scotomata, and visual disturbances. This response is particularly clear after adrenalin infusion, which increases the heart rate and cardiac output. After noradrenalin which contracts the peripheral blood vessels, causing vagal inhibition of the heart rate, such symptoms were less noticeable.

The most typical changes in the blood chemistry after adrenalin infusion were a rise in blood sugar, a rise in lactic acid, and a fall in serum inorganic phosphate, while the other phosphorus fractions showed no appreciable changes. It was of interest in this connection to study the response of the other phosphorus fractions. We also tried to demonstrate whether any typical metabolic changes are elicited in the group of NCA by adrenalin or noradrenalin infusion.

True, the duration of infusion selected for our tests must be considered fairly short from the point of view of observing chemical changes. It is possible that during infusions lasting several hours the stress factors would have appeared more clearly than was the case in our tests (with infusions lasting 40—45 min.). But infusions of very long duration are technically difficult to administer in hospital conditions; on the other hand, adrenalin and noradrenalin under physiological conditions generally produce a very transient effect, like nerve impulses.

Adrenalin infusion accelerates the basal metabolic rate and mobilizes the chemical energy reserves of the organism. Liver glycogen is converted into blood sugar which is phosphorylated; blood inorganic phosphate at the same time decreases and glucose phosphate ester is set free for the use of the muscles where lactic acid is formed.

Noradrenalin, on the contrary, influences the peripheral vascular tone greatly without causing major metabolic changes, and in this respect its mode of action seems more physiological than that of adrenalin which places a strain on the heart and on carbohydrate metabolism.

A comparison of the metabolic changes in NCA and in normal persons is of interest with a view to studying whether any changes occurred, in the regulation of the circulation, and in the carbohydrate and phosphorus metabolism which are closely related to the activity of the hormones concerned. — In the group of NCA the rise in blood sugar following adrenalin infusion seems to be slightly smaller than in the control group. Thus the response of the sugar metabolism in the patients with NCA seems somewhat weaker than in the control subjects. This suggests a fairly slight disturbance in sugar metabolism. No changes typical of NCA were observed in inorganic phosphate or in other phosphorus fractions. This applies also to lactic acid and potassium.

#### SUMMARY

A series of 15 patients with neurocirculatory asthenia (NCA = I) and 12 controls (= II) were studied to determine the effect of continuous intravenous adrenalin infusion on the circulation, the blood chemistry, and on the subjective symptoms; five patients with NCA

(I) and 5 controls (II) were studied to determine the corresponding effects of noradrenalin. The rate of adrenalin and noradrenalin infusion averaged  $0.2 \mu\text{g/kg/min.}$ , the duration of infusion was 40—45 min., and the total dose varied from 0.6 to 0.7 mg.

Adrenalin increased the systolic pressure in the respective groups by 21 per cent (I) and 20 per cent (II), noradrenalin by 8 per cent (I) and 15 per cent (II); only the response to adrenalin was significant.

Adrenalin caused the diastolic pressure to fall by 28 per cent (I) and 33 per cent (II), but noradrenalin raised this pressure by 30 per cent (I) and 26 per cent (II); the diastolic pressure was thus on an average 46 mm Hg higher during noradrenalin infusion than during adrenalin infusion. — The pulse pressure rose after adrenalin administration by 75 per cent (I) and 95 per cent (II), while noradrenalin reduced it by 23 per cent (I) and 24 per cent (II). With respect to all the above changes the NCA and control groups did not differ significantly.

The heart rate response was as follows: adrenalin increased it by 21 per cent (I) and 29 per cent (II), noradrenalin reduced it by 18 per cent (I) and 27 per cent (II). The difference between the changes due to adrenalin and to noradrenalin was more distinct in the control group (42/min.) than in that of NCA (27/min.). There was a statistically significant difference between the two groups.

Adrenalin infusion increased the blood sugar level by 71 per cent (I) and 97 per cent (II), noradrenalin by only 12 per cent (I) and 26 per cent (II). There was a weakly significant difference between the blood sugar responses to adrenalin: the increase was slightly less in the group of NCA than in the control group.

The two groups (I and II) did not differ significantly with regard to the other chemical constituents of blood.

The inorganic phosphorus fraction responded to adrenalin infusion by falling in whole blood 26 per cent (I) and 23 per cent (II), and in plasma 24 per cent (I) and 35 per cent (II); to noradrenalin it responded by falling in whole blood 8 per cent (I) and 5 per cent (II), and in plasma 1.9 per cent (I) and 8 per cent (II). — Neither adrenalin nor noradrenalin infusion produced any typical changes in blood pyrophosphate-P, hexosephosphate-P, diphosphoglyceric acid-P, total acid-soluble P, total phosphorus, and total lipin phosphorus.

Adrenalin increased the blood lactic acid by 35 per cent (I) and 40 per cent (II). Noradrenalin produced no corresponding rise. The serum potassium decreased slightly following adrenalin and rose slightly following noradrenalin administration, but the changes were not significant. Neither adrenalin nor noradrenalin elicited any typical changes in serum total cholesterol and ester cholesterol.

During the adrenalin infusion the patients showed similar subjective symptoms as they had complained of when their history was taken. The symptoms were more pronounced in the group of NCA than in the control group. Tachycardia, restlessness, and tremor of the hands appeared in both groups during adrenalin infusion. In the patients with NCA the following symptoms occurred in addition: nausea, headache, scotomata and visual disturbances, extrasystoles, compression in the cardiac region, trembling of the whole body.

#### REFERENCES

1. CONWAY, E. J.: *The Microdiffusion Analysis*, Lockwood & Son Ltd, London 1947, p. 222.
  2. GOLDENBERG, M., APGAR, V., DETERLING, R., and PINES, K. L.: *J.A.M.A.* 1949:140:776.
  3. HELVE, O.: *Acta Med. Scand.* 1946:125:505.
  4. HELVE, O.: *Acta Soc. Med. Fenn. Duodecim Ser A.* 1946: Fasc. 3, Tom. XXIV.
  5. HELVE, O.: *Acta Med. Scand.* 1947:127:543.
  6. HELVE, O., and PEKKARINEN, A.: *Ann. Med. Exper. et Biol. Fenn.* 1951:29:105.
  7. KALAJA, T., and VESA, A.: *Acta Soc. Med. Fenn. Duodecim. Ser. A.* 1939: Fasc. 1, Tom. XXI.
  8. LILJEDAHL, S—O., and NORLANDER, O.: *Sv. Läk. tidn.* 1951:48:2762.
  9. PEKKARINEN, A., and ARO, LAURI: *Ann. Chir. et Gyn. Fenn.* 1952: 41:69.
  10. PEKKARINEN, A., and HORTLING, H.: *Acta Endocrinol.* 1951:6:193.
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## PLASMA OXIDATION REDUCTION POTENTIAL AND PERCENTAGE OF RETICULOCYTES

by

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(Received for publication August 30, 1952)

Oxidation and reduction belong to the most widespread and vital processes. Many interacting oxidation reduction systems participate, as well known, e.g. in cell and tissue respiration reactions. The systems known to be involved may be characterized by their normal oxidation reduction potential ( $E_o$ ) and an electrometric titration curve. However, in biological naturally occurring fluids this is not possible. The electrometrically determined electrode potential represents the net potential resulting from many systems, all of which are not reversible, interacting with each other. This complicates considerably the measurements because, e.g., of the mostly long time needed for the stabilization of the electrode potential and makes it particularly difficult to interpret the meaning of the differences found in electrode potentials. Since these oxidation reduction reactions are often not reversible, they are called «apparent potentials» (5) instead of «oxidation-reduction potential», which term is reserved for welldefined reversible oxidation reduction systems.

Measurements of apparent oxidation reduction potentials in biological fluids are rather scanty. Aubel and Levy (2) and Friedheim (8) have studied different organs and tissue homogenates and found significant differences in the oxidation reduction potentials and rH values of the liver, brain, spleen, etc.

The oxidation reduction potential of blood and blood plasma has received comparatively little attention. A survey is given by Serejski and Snejerson (21). Brauner and Soru (7) report on investigations concerning anemia. In cardiovascular diseases and pneumonia, a decrease in the potential has been found in severe cases, but none in cases with favourable prognosis (6). Seasonal changes in blood oxidation reduction potential are seen in cases of pulmonary tuberculosis (3). Legresti (13) and Legresti and Semino (14) developed a method for the determination of oxidation reduction potential both *in vivo* and *in vitro*, and report on changes seen in some pathological cases.

Most of the studies mentioned above have the character of orienting experiments, and a more systematic approach obviously would be desirable. Particularly the relation of blood oxidation reduction potential to blood regeneration and anoxia seems worth taking into consideration. As Niedermeier (17), e.g., points out, many substances forming an oxidation reduction system, such as cystin, promote reticulocytosis, when injected into the body. The same is true for ascorbic acid. Furthermore Soru and Brauner (23) have measured the oxidation reduction potential in the bone marrow of normal and anemic rabbits and noted after repeated bleedings a consistent rise in the potential related to the decrease in the erythrocyte count. Since more and more evidence is accumulating against the concept of direct stimulating effect of decreased oxygen tension on the activity of bone marrow (1, 10, 16, 24), a change in blood oxidation reduction potential in anoxic and anemic conditions might be a useful guide in search for the humoral stimulus of blood regeneration.

In view of these facts, we thought it worth while to investigate whether in conditions with increased blood regeneration plasma apparent oxidation reduction potentials show any measurable differences. As a sign of increased regeneration, the degree of reticulocytosis following blood loss was considered. This seemed motivated particularly because, according to Oliva and Frascarelli (18), the increased amount of reticulocytes in peripheral blood forms a condition for demonstration of erythropoiesis stimulating substances in the bloods of anemic subjects.

Preliminary reports on this paper were published in 1951 (11, 20).

## MATERIAL AND METHODS

For the experiments, rabbits weighing between 2.2 and 2.7 kg were used throughout. In order to standardize the conditions, the diet was carefully kept constant. It consisted of 300 g of barley, 75 g of oats and 3 g of pine needles per day and liberal water supply. Every experimental series was made by bleeding the rabbits 4—6 times with 2—3 days' intervals for some three weeks. The amount of blood taken varied between 12—18 ml. Blood specimens for reticulocyte counts were taken at 9 o'clock a.m., the blood sample for oxidation reduction potential and pH measurements between 10 a.m. and 1 o'clock p.m., before the rabbits were fed. The method of bleeding was at first cardiac puncture, but since it was considered necessary always to get arterial blood, this procedure often terminated in heart tamponade and death of the animal. In later experiments we therefore prepared a carotid loop in the rabbit and punctured the exteriorized left carotid artery. Between the operation and the actual use of the rabbit for the experiments several weeks were interposed. In the beginning of a series the arterial loop puncture did not cause any apparent discomfort to the animal, but after several punctures the carotid obviously became tender and the rabbits got excited by the puncture. A longer rest period was then necessary, and the continuation of the experimental series was not always possible.

For the determination of the apparent oxidation reduction potential of arterial plasma a special method was developed, and will be published in detail later on (19). The principle of the method was as follows: in a glass bulb in which an electrode and an agar-KCl-bridge were fitted, a glass capillary could be inserted below and a syringe above the bulb. The bulb could thereby be filled with plasma by suction with help of the syringe. As electrode, a platinum plate electrode and as reference electrode a saturated calomel KCl electrode were used. The blood sample was taken anaerobically in a Luer syringe sealed with mineral oil, and the blood injected below a layer of mineral oil in a centrifuge tube. Immediately after the puncture the blood sample was centrifuged, and the clear plasma was transferred into the measuring bulb. Thus the *measurements* were made *in the O<sub>2</sub> gas tension existing in the plasma*. For potential measurements a valve-potentiometer »Radiometer», type PHM 22 b was used. The potential was read after 10 min. stabilization time. Of every sample 2—3 parallel determinations were made. To check the stability of the electrodes a potential reading with the bulb filled with phosphate buffer pH 6.8 was made. The mean potential thus recorded was  $284 \pm 5.6$  or  $529 \pm 5.6$  mV corrected to normal hydrogen electrode potential. In a part of the experiments immediately after the oxidation reduction potential determination, the plasma pH was measured electrometrically with the same apparatus but with a glass electrode instead of the platinum plate electrode.

The reticulocytes were counted in dry preparations stained with giemsa and made as usually with a drop of 1 per cent brilliant cresyl-blue solution

TABLE 1

Exp. Series	Rabbit No	Date 1951	Reticulo-cyte %	Plasma potential		pH	Room Temp. °C
				E <sub>cal</sub> *	E <sub>h</sub>		
I. 1.	A 735	14.4	4.6	-13.5	231	—	
2.		17.4	4.3	-18.5	226	—	
3.		21.4	10.4	+13.5	259	—	
4.		23.4	17.4	+19.0	264	—	
5.		25.4	16.0	+17.0	262	—	
II. 1.	206	18.4	6.1	- 8.5	236	—	
2.		23.4	12.2	+16.5	262	—	
3.		26.4	11.2	+13.0	258	—	
4.		5.5	4.0	+ 3.0	248	—	
5.		11.5	7.3	- 4.5	240	—	
III. 1.	O. M.	20.4	6.2	- 3.5	241	—	
2.		24.4	12.2	+33.5	279	—	
3.		30.4	9.2	+18.5	264	—	
4.		9.5	3.8	+12.0	257	—	
IV. 1.	206	8.6	1.0	-21.5	223	—	20.1
2.		11.6	4.2	-35.5	209	7.67	19.9
3.		13.6	8.5	-14.0	231	7.68	20.1
4.		15.6	8.6	-13.5	231	7.62	20.0
5.		18.6	11.8	—	—	7.63	20.2
6.		20.6	15.7	- 3.5	241	7.64	19.5
7.		4.7	5.1	- 5.0	240	7.68	20.9
V. 1.	O. M.	11.6	4.8	-31.0	214	7.67	19.7
2.		13.6	6.5	-33.0	212	—	19.5
3.		15.6	11.1	- 5.5	239	7.60	20.0
4.		18.6	10.2	-14.0	231	7.59	20.0
5.		4.7	3.0	—	—	7.60	20.7
VI. 1.	215	9.6	3.5	-36.0	209	—	20.0
2.		12.6	4.3	-34.5	210	7.66	19.4
3.		14.6	8.6	- 8.0	237	7.63	20.0
4.		19.6	11.1	—	—	7.60	20.3
5.		3.7	3.1	-32.0	213	7.65	20.4
VII. 1.	214	22.6	1.6	-35.5	209	7.62	19.8
2.		26.6	1.8	-28.5	216	7.60	21.6
3.		28.6	5.2	- 7.0	238	7.54	21.1
4.		2.7	13.9	-12.0	233	7.64	20.9
5.		4.7	22.7	—	—	7.70	20.6
VIII. 1.	222	27.6	1.4	-38.0	207	—	21.8
2.		29.6	3.4	-16.5	228	7.61	20.9
3.		2.7	9.1	—	—	7.60	21.0

\* Average of two parallel determinations.

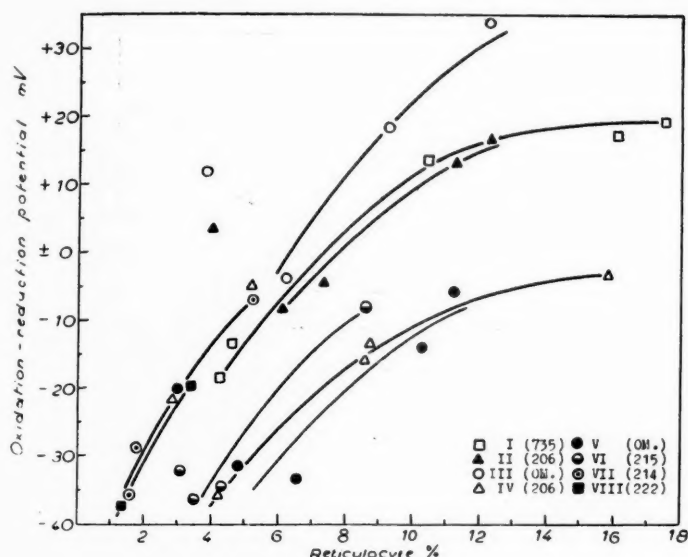


Fig. 1. — Oxidation reduction potential and reticulocyte percentage. Roman numbers refer to the experimental series (Table 1).

added to a drop of blood. Twice or three times 1,000 cells (2—3 different slides) were counted. 6 rabbits and 8 experimental series were analysed in this way.

#### RESULTS

The results are seen in Table 1 and in Fig. 1, in which the apparent oxidation reduction potential referred to the calomel electrode potential and given in mV is plotted against the reticulocyte percentage. The values in Table 1 are also given as  $H_2$ -electrode potentials ( $E_h$ ).

The experimental series II and IV are made with the same rabbit, as in series III and V. The difference between the values of the two series is probably due to the fact that different platinum electrodes were used in these two series. As may be seen, the potential increases as the reticulocyte count rises from 3—5 per cent to 8—10 per cent. Between these reticulocyte values the increase in the potential is some 30 mV. Within this range of reticulocyte percentages the steepest rise in the potential is seen to cover 2/3 of

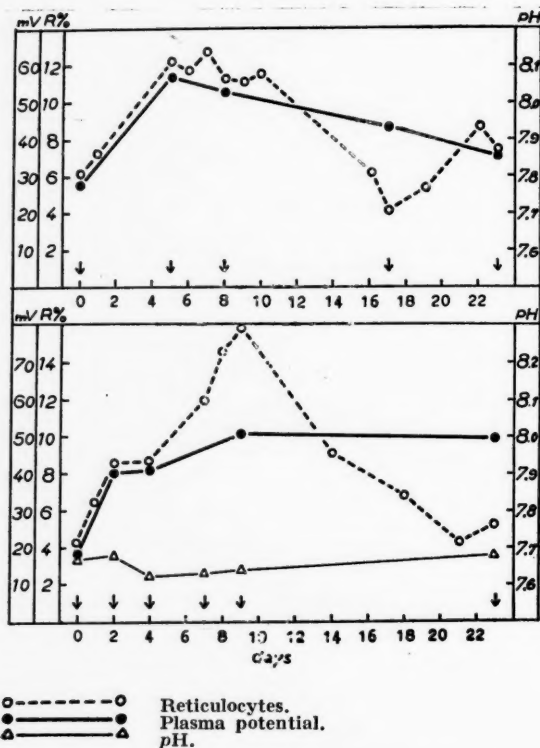


Fig. 2. — Reticulocyte percentage, plasma potential and pH plotted against time in days. Up: Series No II. Below: Series No IV. Rabbit No 206.

the total increase (40—45 mV). A further increase in the reticulocyte percentage leads only to a slight further rise of the potential, the slope of the curve thus decreasing conspicuously. Furthermore, the values which correspond to the same series fall on the same curve, whereas the series as a whole may differ with respect to its position on the chart. Only two values fall clearly outside the range of the curves.

#### DEVELOPMENT OF RETICULOCYTOSIS AND ALTERATIONS IN PLASMA POTENTIALS DURING REPEATED BLEEDINGS

During the experiments came out, that the proportionality between plasma potential and reticulocytosis was most satisfactory in the beginning of each series and each rabbit, but less so at the

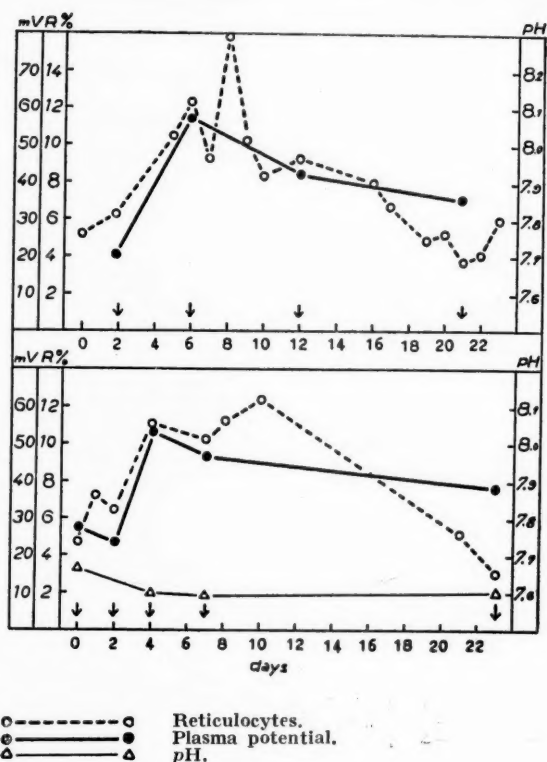


Fig. 3. — Reticulocyte percentage, plasma potential and pH plotted against time in days. Up: Series No III. Below: Series No V. Rabbit 0. M.

end of the series. It seemed therefore necessary to compare the reticulocytosis and plasma potential as a function of time. The following charts (Figs. 2—4) show the initial proportionality in a striking way. In order to make the two variables commensurate a 2 per cent increase in the reticulocyte count was made to correspond to a 10 mV increase in the oxidation reduction potential, and the reticulocyte percentage 0 to correspond to the zero potential. Fig. 2 illustrates two experimental series performed on the same rabbit. The experimental period was 23 days. As will be seen, at the beginning of the series the two curves overlap each other exactly. This holds true also for Figs. 3 and 4. On the other hand, it is obvious that in a later phase of the experimental period the potential does not follow the course of the percentage of reticulo-

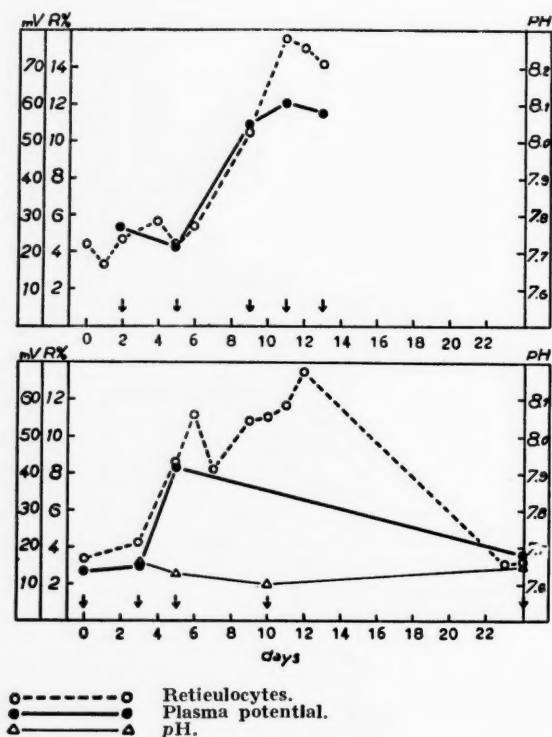


Fig. 4. — Reticulocyte percentage, plasma potential and pH plotted against time in days. Up: Series No I. Rabbit No 735. Below: Series No VI. Rabbit No 215.

cytes any more. The scattered values seen in Fig. 1 refer to those determinations which were made in the latest phase of the experimental series. The higher potential recorded in this phase depends obviously on the fact that the potential is not normalised as quickly as the reticulocyte count. Consequently, during recovery from blood loss, the decrease of reticulocyte percentage is steeper than that of the potential.

#### OXIDATION REDUCTION POTENTIAL OF ARTERIAL PLASMA AND HYDROGEN ION CONCENTRATION

In most cases the pH of the plasma was determined. All values fell within pH 7.59–7.70, measured in room temperature (20° C). The lower charts in Figs. 2, 3, and 4 show the values of pH. As seen,

the pH remains rather stable and is not affected by the bleeding. Obviously variations in the oxidation reduction potential cannot be explained by variations in the pH of arterial plasma (Table 1).

#### DISCUSSION

The conclusion we may reach on basis of the experiments reported in the foregoing is that a reticulocytosis caused by bleeding of the animal leads to an increase in the apparent oxidation reduction potential of arterial plasma. This result is in good agreement with those of Soru and Brauner (23), who measured the potential in the bone marrow of anemic rabbits. It is most probable that the introduction of electrodes in the bone marrow causes a hemorrhage within the marrow so that the potential recorded possibly represents the potential of blood as well.

If the results are studied from a methodological point of view the part played by the properties of electrodes inducing possibly a systematic error must be considered. Some observations seem to suggest that only values recorded with the same electrode are strictly comparable. However, the difference in the level of the potential is not much different even when the measurements are made with different electrodes. Unfortunately, it has not been possible to determine the absolute value of this systematic error because only one electrode at a time was at our disposal. Anyhow, the reproducibility of our results indicates that the technical difficulties of recording of plasma apparent redox potentials are at least partly eliminated. It must be pointed out, however, that this result is obtainable only by extreme thoroughness in the manipulation of the apparatus, the handling of electrodes and in standardizing the experimental conditions as to the state of the animal. It is well conceivable that if the animal becomes excited during arterial puncture, adrenalin may be mobilized and, as an oxidation reduction system, adrenalin may alter the equilibrium of the oxidation reduction systems influencing the apparent potential of the plasma. This may be partly responsible for the diverging values encountered in the latest phase of an experimental series. The methodological details and the experimental errors in duplicate determinations will be given in a later paper (19).

What biological value can now be given to the fact that reticulocytosis caused by bleeding leads to an increase in the arterial plasma potential, the hydrogen ion concentration remaining unaltered? Of those substances involved in the reproduction of an equilibrium between the various oxidation reduction systems the following may be mentioned: adrenalin, ascorbic acid, cystein, glutathione, methemoglobin and different oxido-reductases catalyzing oxidation-reduction reactions. Every alteration in the concentration of their oxidized and reduced forms may involve an alteration in the apparent oxidation reduction potential of the arterial plasma.

Since the measurements according to the procedure adopted in this study are made in arterial plasma with free molecular oxygen dissolved in it, the potential recorded does not allow any comparison to the normal potential of different known oxidation systems. The presented data only suggest that the blood loss and the possibly induced tissue anoxia upsets the normal physiological balance between these systems. This disequilibrium may lead to increased output of reticulocytes and to a series of oxidation reduction reactions, the net effect of which is mirrored in an alteration in the apparent oxidation reduction potential of the plasma. Indications of the existence of chemical alterations are actually seen. Litarczek and co-workers (15) have found an increase in cellular glutathione (reduced SH-form) in anemia due to repeated bleedings (5 rabbits). According to Bichel (4), reduced glutathione content per erythrocyte increases after bleeding. Since no determinations of the oxidized form were performed, there is no information obtainable about the relation between reduced and oxidized forms. Bichel was not able to demonstrate any relation between glutathione (SH-form) value, and reticulocyte count. Galeone, Levi and Segre (9) have determined the relation between dihydrodiphosphopyridine-nucleotide (DPN-H<sub>2</sub>) to its dehydrated form (DPN) in cardiac decompensation and noted a decrease of the ratio  $\frac{\text{DPN-H}_2}{\text{DPN}}$ .

Whether the decompensation had led to increased reticulocyte percentage, as it often does, was not ascertained. Krasno and co-workers (12) report on an increased utilisation of ascorbic acid in subjects exposed repeatedly to 18,000 feet simulated altitude. The nature of the processes resulting in these chemical alterations are not known. There is one common trait in all of these different

findings concerning most variable hypoxic conditions: a relative decrease in reducing substances. One could therefore in all these conditions expect an increased oxidation reduction potential of the blood. In hemorrhagic anemia this has been substantiated, but, e.g., in decompensated heart disease the results of Bogdanova (6) point in the reverse direction, and of anoxic hypoxia as yet no information is available. The chemical alterations in hemorrhagic anemia are going on in this laboratory (P).

## SUMMARY

1. The study concerns electrometric determinations of oxidation reduction potential in arterial plasma of anemic rabbits.

2. Determinations before and during recovery from blood loss show that in the rabbit the arterial blood plasma potential is dependent upon the intensity of regeneration of blood, the oxidation potential increasing with the increasing reticulocyte percentage. When the reticulocyte percentage rises from 3—5 to 8—10, the oxidationreduction potential shows an increase of about 30 mV. This covers about  $\frac{2}{3}$  of the total potential increase.

3. Proportionality between reticulocyte percentage and plasma potential is clearest at the beginning of reticulocytosis.

4. Changes in pH-values during reticulocytosis are negligible.

5. The possible role of plasma potential level in blood regeneration is discussed.

## REFERENCES

1. ASTALDI, G., BERNARDELLI, E., and REBAUDO, G.: *Experientia* 1952:8:117.
2. AUBEL, E., and LEVY, R.: *Ann. Physiol.* 1931:7:447.
3. BECKMAN, P.: *Dtsch. med. Wschr.* 1939:65:507.
4. BICHEL, J.: *Acta Med. Scand.* 1944:117:474.
5. BLADERGROEN, W.: *Physikalische Chemie in Medizin und Biologie*, Basel 1945.
6. BOGDANOVA, A. D.: *Klin. med.* 1938:16:1372.
7. BRAUNER, R., and SORU, E.: *C.r. Soc. biol.* 1934:116:1176.
8. FRIEDHEIM, E. A. F.: *C.r. Acad. Sci.* 1929:189:266.
9. GALEONE, A., LEVI, E., and SEGRE, G.: *Acta Med. Scand.* 1951:139:308.
10. GRANT, W. C., and ROOT, W. S.: *Feder. Proc.* 1947:6:114.

11. JALAVISTO, E., and PIHA, S.: *Acta Physiol. Scand.* 1951. 25. Suppl. 89. p. 43.
  12. KRASNO, L. R., CILLEY, J. H., BOUTWELL, J. H., IVY, A. C. and FARMER, M. S.: *J. Aviat. Med.* 1950:21:283.
  13. LEGRESTI, L.: *Acta Arg. Fisiol. Fisiopat.* 1950:1, 21, 51, 59, 67.
  14. LEGRESTI, L., and SEMINO, O. E.: *Acta Arg. Fisiol. Fisiopat.* 1950:1:75.
  15. LITARCZEK, G., AUBERT, H., COSMULESCO, I., and NESTORESICO, B. *C.r. Soc. biol.* 1931:106:110.
  16. MAGNUSSEN, J. D.: *Acta Pharmacol. et Toxicol.* 1949:5:153.
  17. NIEDERMEIER, S.: *Ärzt. Forschung* 1949, 3, II, 149.
  18. OLIVA, G., and FRASCARELLI, R.: *Rif. med.* 1946:60:437.
  19. PIHA, S.: To be published.
  20. PIHA, S.: *Farmaceutisk Notisblad* 1951:60:213.
  21. SEREJSKI, M., and SNEJERSON, S.: *Z. ges. exper. Med.* 1937:100:621.
  22. SEYDERHELM, R.: *Die Hypovitaminosen*, Leipzig 1938.
  23. SORU, E., and BRAUNER, R.: *C.r. Soc. biol.* 1931:107:426.
  24. WARREN, Ch. O.: *Am. J. Physiol.* 1941:133:482 P.
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## EFFECT OF NICOTINE ON SECRETION OF DUODENAL GLANDS IN DOG

by

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(Received for publication September 2, 1952)

The effect of smoking on gastric function and its possible bearing on peptic ulcer is a subject of great importance, although still an object of confusing discussion. Studies to elucidate these relationships, however, seem to be indicated in view of the clinical observations that a great number of ulcer patients appear to be improved by avoiding tobacco and also in view of some well-designed studies in which it has been stated that smoking decreases the effectiveness of the antacid management of the peptic ulcer patient (16).

Regardless of whether smoking or nicotine has any etiologic bearing on the ulcer disease or not, it was thought to be worth while to study the effect of nicotine on the function of the duodenal (Brunner's) glands, since it has been suggested (5, 6, 8, 9) that these glands serve as a protection against mechanical and chemical irritants by virtue of their alkaline, highly viscous mucus secretion.

### MATERIAL AND METHODS

So far there are no means to study the function of the duodenal glands in man. Such studies, however, have been possible on animals whose digestive tracts and secretory functions have been shown to be closely similar to those of man, viz. on the cat and dog. These methods have been introduced by Florey and Harding (5, 6) and later improved by Sonnenschein, Grossman and Ivy (14) by preparing chronic duodenal pouches in dog. The operative

procedures in preparing such pouches consist of the fashioning of a pouch of the suprapapillary portion of the duodenum and of bringing this through an abdominal stab-wound and suturing it to the abdominal wall. The vascular pedicle of the segment is kept intact. Continuity of the intestine is re-established by end to side gastro-duodenostomy. For some studies the excised portion of the duodenum is cut lengthwise along the antimesenteric edge and sutured to the abdominal wall in the form of a rectangular flap. In both cases the excised part of the duodenum brought out of the abdominal cavity allows the direct collecting of the juice secreted by the glandular elements of the region. In previous studies it has been found that this secretion mainly presents the product of Brunner's glands, which have been demonstrated to have both nervous and humoral control (5, 6).

Preparations of the flap type were used in the present study. The secretion was collected by means of a funnel which led to a graduated centrifuge tube. The amount of juice secreted could thus be measured directly. In these studies only the amount of juice secreted was considered.

The experiments were carried on 4 mongrel dogs. Before each experiment they were first allowed to stand in wooden enclosures one hour before the observations were begun. This was considered essential in order to avoid the effect of bringing the dogs from their cages to the laboratory, the effect of the manipulations when placing the funnel and tube, etc. Care was taken to avoid the rubbing of the exposed flap by the funnel and also that the animals were not able to remove or touch the preparations.

After the resting period the juice was collected during 5 hours and the amounts recorded every hour. The first two hours present the basal secretion; at this point the animals were given their daily test meal. The amount of postprandial secretion exceeds that of the basal secretion and presents that phase of secretion which is under humoral control. The extent of the increase of the secretion depends on several factors. One main factor in this kind of experimentation is the type of duodenal preparation employed. As has been shown by Blickenstaff, Grossman and Ivy (1), the preparation of the flap type responds much less to a test meal than the pouch does. The contents of the test meal might also be of some importance. In these experiments the following diet was

used: 200 ml of milk mixed with 200 grams of ground beef meat. The meal was thoroughly mixed by means of an electrical stirrer. All of the animals ate their meal voluntarily within a few minutes.

The nicotine was administered by subcutaneous injection.

### RESULTS

The effect of a single dose of nicotine was first studied in each dog. After establishing the control levels of the two secretory phases in each animal during daily observations over a period of two weeks, 1.0 mg of nicotine was administered to each of them. The results are shown in Table 1.

TABLE 1

EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF 1.0 MG OF NICOTINE ON THE DUODENAL SECRETION. THE ASTERISK DENOTES THE TIME OF INJECTION. THE CONTROL VALUES REPRESENT THE MEAN SECRETION DURING THE CONTROL PERIOD

Dog No.		cc of Juice Secreted during the					Result
		1 st	2 nd	3 rd	4 th	5 th h.	
1	control	1.6	1.3	1.6	1.8	1.9	initial increase
	after nicotine	*1.7	2.2	1.8	1.7	1.6	
2	control	2.9	2.6	3.2	3.5	3.4	no effect
	after nicotine	3.1	*2.9	3.0	4.4	3.6	
3	control	3.1	2.6	4.0	4.1	3.7	no effect
	after nicotine	3.0	3.3	*3.8	4.3	4.0	
4	control	2.7	3.0	3.5	3.0	2.1	decrease
	after nicotine	2.8	*1.8	1.3	0.8	0.5	

The results seem to indicate that the alkaloid had no consistent effect on the secretion. In dogs 1, 2 and 3 it did not markedly alter the usual secretory pattern. The slight stimulation observed during the second hour in dog 1 was not present in the other animals. In dog 4 the injection of nicotine resulted in a marked decrease in the secretion. Fig. 1 illustrates graphically the secretory pattern in one dog (No 2).

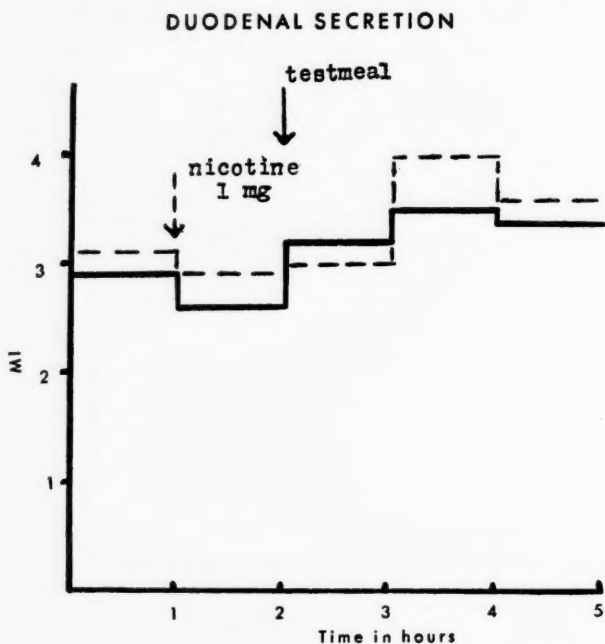


Fig. 1. — Effect of a single subcutaneous injection of 1.0 mg of nicotine on the duodenal secretion. The solid line represents the mean output during the control period, the dotted line represents the output in the nicotine experiment. (Dog No 2).

TABLE 2

EFFECT OF 8 DAYS DAILY ADMINISTRATION OF 1.0 MG OF NICOTINE ON THE DUODENAL SECRETION. A REPRESENTS THE MEAN VALUES OF THE CONTROL PERIOD, B THE VALUES OF THE FIRST HALF OF THE NICOTINE PERIOD, C THE SECOND HALF AND D THE MEAN VALUES OF THE FIRST 4 DAYS AFTER THE NICOTINE ADMINISTRATION, E AVERAGE OF 4 DAYS SECRETION 10 DAYS LATER

Dog No.	A	A	B	C	D	E
1	Basal secretion	3.0 ± 0.6	2.2 ± 0.5	1.8 ± 0.7	1.7 ± 0.2	2.8 ± 0.4
	After-meal secretion	3.5 ± 0.5	3.4 ± 0.6	2.6 ± 0.7	2.6 ± 0.6	3.0 ± 0.4
2	Basal secretion	3.2 ± 0.7	2.5 ± 0.6	2.2 ± 0.6	1.4 ± 0.3	4.1 ± 0.6
	After-meal secretion	4.0 ± 0.4	3.6 ± 1.0	2.6 ± 0.2	2.4 ± 0.4	3.3 ± 0.5

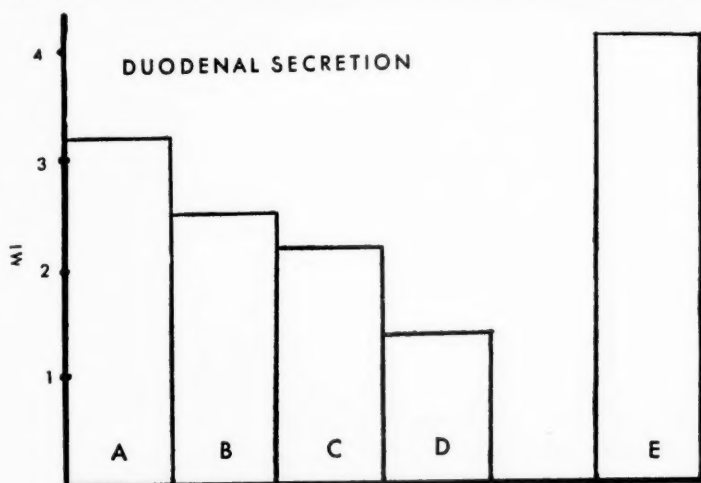


Fig. 2. — Mean hourly basal secretion in dog No 1 before (A), during (B and C) and after (D and E) daily subcutaneous administration of 1.0 mg of nicotine. A represents the mean values of 10 days, B, C, D and E each the mean values of 4 days. Time interval between D and E is 10 days.

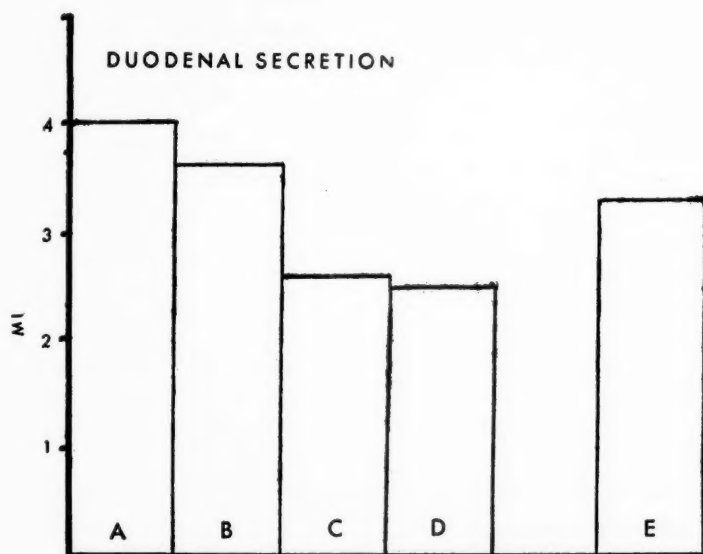


Fig. 3. — Mean hourly after-meal secretion in dog No 1 before (A), during (B and C) and after (D and E) daily administration of 1.0 mg of nicotine. A represents the mean values of 10 days, B, C, D and E each the mean values of 4 days. Time interval between D and E is 10 days.

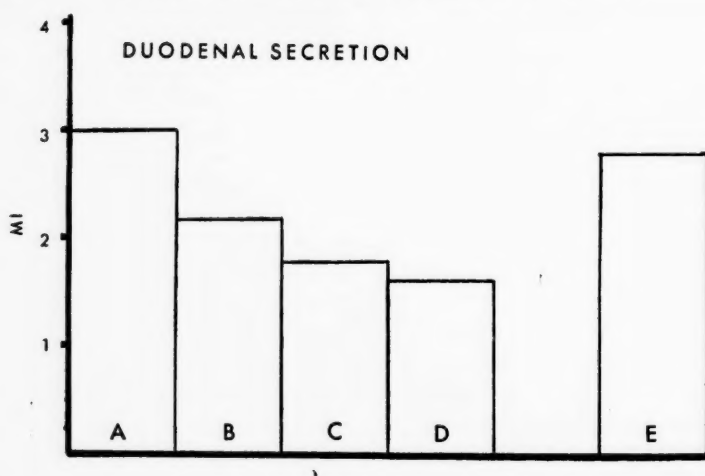


Fig. 4. — Mean hourly basal secretion in dog No 2 before (A), during (B and C) and after (D and E) daily subcutaneous administration of 1.0 mg of nicotine. A represents the mean values of 10 days, B, C, D and E each the mean values of 4 days. Time interval between D and E is 10 days.

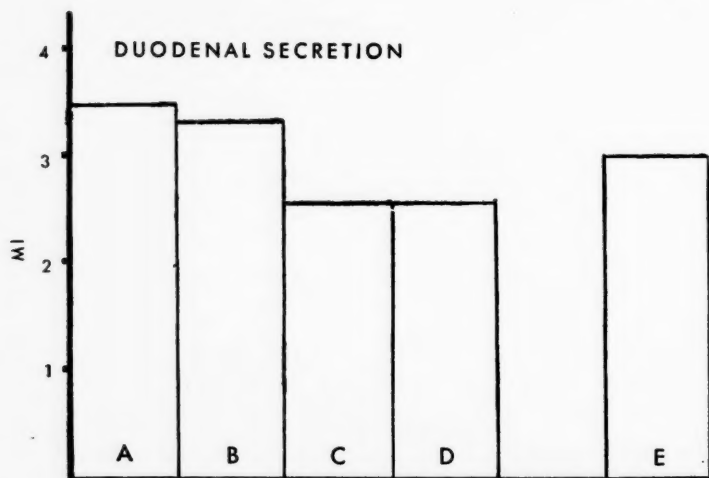


Fig. 5. — Mean hourly after-meal secretion in dog No 2 before (A), during (B and C) and after (D and E) daily subcutaneous administration of 1.0 mg of nicotine. A represents the mean values of 10 days, B, C, D and E each the mean values of 4 days. Time interval between D and E is 10 days.

The effect of prolonged nicotine administration was then studied in two dogs (Nos. 2 and 3). After the control secretion was established, the dogs received 1.0 mg of nicotine daily over a period of 8 days. This procedure did not result in any general reaction such as nausea, loss of weight, or diarrhea; neither did the animals lose their appetite. The gross appearance of the juice did not change and remained highly viscous to the end of experimentation.

The results are listed in table 2 and illustrated graphically in Figs. 2, 3, 4 and 5. In the illustrations, the basal secretion (Figs. 2 and 4) and the after-meal secretion (Figs. 3 and 5) are treated separately.

It will be seen that the first 4 days of the administration of nicotine (phase B) did not markedly effect the amount of secretion. The further administration (phase C) of the poison resulted, however, in a gradual reduction of the output. This reduction continued for a certain period after the injections of nicotine were interrupted (phase D). Thus the lowest secretory levels were observed during the first 4 days without nicotine treatment and comprised only 45—60 per cent of the control values for the basal secretion and 60 to 70 per cent for the after-meal secretion. By applying the t-test (15), it was found that the mean values listed in period D in each case and dog indicated a statistically highly significant reduction.

The observations were continued for another fortnight. At this time the output by the duodenal glands gradually increased, so that at the end of this period it had reached the original control level.

#### DISCUSSION

The results seem to indicate that nicotine diminishes the output of the duodenal glands. To what extent the reduction would proceed if the nicotine administration were continued over longer periods deserves further investigation. The amount of nicotine used in these experiments corresponds roughly to the amount of the alkaloid absorbed from the mouth and lungs by smoking one cigarette of medium strength (13). Attempts were also made to study the direct effect of smoking on duodenal secretion. By applying masks, the dogs were forced to inhale smoke from a cigarette. It appeared, however, to be very difficult to perform a long range of studies by

this means since the discomfort caused by the smoking distempered the dogs to such extent that no reliable results could be obtained. Since the avoidance of psychic effects on the results is of utmost importance in experiments of this kind, further studies on these lines were not conducted.

As to the mechanism of the reduction observed, only suggestive remarks can be made. Nicotine and smoking have been claimed to cause alterations in the gastric and intestinal motility (2, 3, 7, 11, 12). The explanation that the change in the motility of the excised duodenal segment mediates the reduction in the glandular secretion does not seem to be plausible. The preparation of the flap type was used in these experiments and the secretion from this type of preparation has shown to be little effected by changes in duodenal motility (1). On the other hand it is known that nicotine causes paralysis of the autonomic ganglia. It might be the stimulatory effect carried by the parasympathetic innervation of the duodenal glands which is abolished by nicotine.

#### SUMMARY

The effect of subcutaneously administered nicotine in doses of 1.0 mg was studied on dogs prepared with chronic duodenal fistulas (flap type).

A single dose of nicotine did not have a consistent effect on the duodenal (Brunner's) secretion.

Prolonged administration of nicotine in doses of 1.0 mg once in a day over a period of 8 days resulted in a gradually increasing reduction of the duodenal secretion. The secretion reached the original control level in two weeks after the last nicotine injection.

#### REFERENCES

1. BLICKENSTAFF, D., GROSSMAN, M. I., and A. C. IVY: *Am. J. Physiol.* 1949:158:122.
2. CARLSON, A. J., and J. H. LEWIS: *Am. J. Physiol.* 1914:34:149.
3. DANIELPOLU, D., SIMICI, D., and C. DIMITRIU: *C. R. Soc. Biol., Paris.* 1925:92:535.
4. FISCHER, R. A.: *Statistical Methods for Research Workers.* Seventh edit., Edinburgh 1938.
5. FLOREY, H. W., and H. E. HARDING: *J. Path. Bact.* 1933:37:431.
6. FLOREY, H. W., and H. E. HARDING: *J. Path. Bact.* 1934:39:255.

7. GRAY, I.: Ann. Int. Med. 1929—30: 3:267.
  8. HARTIALA, K., IVY, A. C., and M. I. GROSSMAN: Am. J. Physiol. 1950:162:110.
  9. HARTIALA, K., MAGEE, D. F., and M. I. GROSSMAN: Am. J. Physiol. 1950:163:34.
  10. IVY, A. C., GROSSMAN, M. I., and W. H. BACHRACH: Peptic Ulcer. Blakiston Co. Philadelphia 1950.
  11. MULINOS, M. G.: Proc. Soc. Exp. Biol. Med. 1927:25:49.
  12. SCHNEDORF, J. G., and A. C. IVY: J. A. M. A. 1939:112:898.
  13. SOLLMAN, T.: A Manual of Pharmacology. Saunders Co. Seventh edition. Philadelphia 1950.
  14. SONNENSCHN, R. R., GROSSMAN, M. I., and A. C. IVY: Acta Med. Scand. 1947:196:295.
  15. »STUDENT»: Biometrika 1908:6:1. — Quoted by FISCHER.
  16. REWITZ, E., and H. FUSS: Die Pathogenese des peptischen Geschwürs des Magens und der oberen Darmabschnitte. Stuttgart, Ferdinand Enke 1928. — Quoted by IVY *et al.*
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## EFFECT OF CERTAIN SUTURE MATERIALS ON THE RESPIRATORY TRACT

### EXPERIMENTAL STUDY

by

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(Received for publication September 5, 1952)

We regret that it has not been possible for us to contribute a paper on medical chemistry to the present homage volume of Professor P. E. Simola; instead, we present a brief report on a series of experiments carried out to study the effect of certain suture materials on living tissues. The starting point of the study was the fact that so far no unanimity has been reached as to what is the best suture material for the larger respiratory passages, such as the trachea and the main bronchi. The subject of suture materials as such is quite comprehensive, and we are not going to touch here upon earlier publications on it. It will suffice to say that materials for suturing respiratory passages have to meet special requirements. Every worker in the field of thoracic surgery knows, for example, that the strength of bronchial suture is of decisive importance for successful resection. One of the things to be taken into consideration in choosing the suture material is the bacterial content of the respiratory tract. Recently, bronchial fistula has occasionally been present after resection for pulmonary tuberculosis, which is now quite frequent in the treatment of that disease. It is this that has brought the question of the strength of suture much to the fore. Even when the resection stump is covered with living tissue, such

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as the pleura, the formation of a fistula cannot always be efficiently avoided. Every surgeon, of course, is anxious to choose the best possible suture material for his cases. In order to obtain a notion of the relative values of some common suture materials, we tried to find out —

1) whether any of the suture materials to be tested had any bacteriostatic effect;<sup>1</sup>

2) what kind of tissue reactions the various suture materials produced, especially in the tissues of the respiratory tract; and

3) how a tuberculous infection in bronchial tissue behaved with respect to different suture materials.

#### TECHNIQUE AND RESULTS

*Bacteriostatic Effect.*<sup>2</sup>—In a series of tests *Staphylococcus aureus* or *Escherichia coli* was inoculated in Fraenkel's medium; in this suspension were placed bits of equal length of the following suture materials: silk (No. 3), nylon (No. 3), steel wire ( $\phi$  0.05 mm), tantalum wire ( $\phi$  0.05 mm), silver-plated and gold-plated steel wire ( $\phi$  0.16 and 0.01 mm). The growth of the bacterial flora in the suspension was watched for six days. The results are shown in table 1.

TABLE 1  
BACTERIOSTATIC EFFECT OF SOME SUTURE MATERIALS IN FRAENKEL'S CULTURE MEDIUM

	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		
	1 Day	2 Days	6 Days	1 Day	2 Days	6 Days
Gold-plated steel wire, $\phi$ 0.16 mm	—	—	+	+	+	+
Gold-plated steel wire, $\phi$ 0.01 mm	—	—	+?	—	+	+
Silver-plated steel wire, $\phi$ 0.16 mm	—	—	—	—	—	—
Silver-plated steel wire, $\phi$ 0.01 mm	—	—	—	+?	+	+
Steel wire, $\phi$ 0.05 mm	—	+?	+	+?	+	+
Tantalum wire, $\phi$ 0.05 mm	—	—?	+	—?	—?	—?
Silk No. 3	—	—	—?	—	—	+
	Control culture: positive			Control culture: positive		

<sup>1</sup> We refer to the studies carried out by Alha and Koulumies on the effect of various metals on different bacterial strains.

<sup>2</sup> We are indebted to Prof. K. O. Renkonen, M. D., Director of the University Department of Serology and Bacteriology, Helsinki, who in a most obliging way placed his experience in bacteriological matters at our disposal.

TABLE 2  
BEHAVIOUR OF TRACHEAL TISSUE IN THE PRESENCE OF SUTURE

<i>Catgut</i>	<i>Silk</i>	<i>Nylon</i>	<i>Mersilene</i>	
10 days. — Granulation tissue around suture rich in blood vessels, edematous. Numerous leukocytes and histiocytic cells. Juvenile fibroblasts. Numerous foreign body giant cells. Subchondral abscess in one specimen (Fig. 1 a).	Granulation tissue poor in blood vessels; moderate edema. Numerous histiocytes and foreign body giant cells. In the periphery of granuloma, collagenic connective tissue fibres. A few leukocytes and lymphocytes (Fig. 3).	Granulation tissue edematous. Moderate number of leukocytes and lymphocytes. Moderate vascularisation. Histiocytes and foreign body giant cells. Numerous fibroblasts.	Granulation tissue rich in mature fibroblasts and collagen fibres, poor in lymphocytes. A few foreign body giant cells in periphery. Slight edema.	Fibrin Granu slight Numer proble phocy vessel body
20 days. — Granulation tissue edematous. Decreased vascularisation. Moderate number of leukocytes. Fibroblasts. Foreign body giant cells. Peripheral collagenic connective tissue (Fig. 1 b).	Granulation tissue poor in blood vessels; no edema. Foreign body giant cells phagocytising the suture material. A few lymphocytes.	No edema. No inflammatory cells except a few lymphocytes. Scar tissue still rich in cells. No foreign body giant cells.	In the periphery of granuloma, mature connective tissue; in the centre, looser connective tissue. Numerous fibroblasts. A few lymphocytes and foreign body giant cells.	Scar of m tissu cells cells No cells
40 days. — Granulation tissue consisting mainly of mature fibroblasts and collagenic connective tissue fibres. Poor in blood vessels. Leukocytes still present. Foreign body giant cells. Submucous abscess in one specimen (Fig. 1 c).	No inflammatory cells. Foreign body giant cells phagocytising the suture material. Mature connective tissue.	Granulation tissue organised, poor in cells. Occasional lymphocytes.	Scar made up of firm connective tissue, poor in cells. Some juvenile fibroblasts still in centre.	No tion of r tissu

TABLE 2  
OF SUTURES MADE WITH DIFFERENT MATERIALS

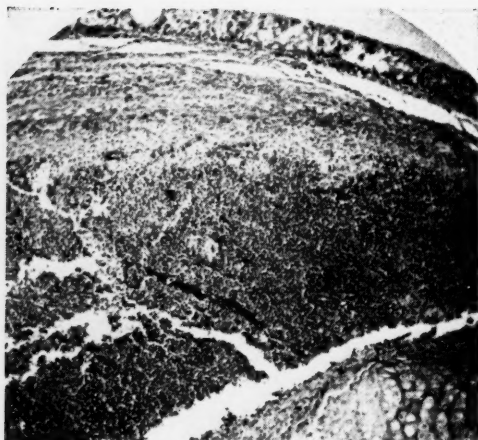
	<i>Steel Wire</i>	<i>Tantalum</i>	<i>Silver-plated Steel Wire</i>	<i>Gold-plated Steel Wire</i>
tissue ure fi d col poor tes. A body s in Slight	Fibrin next to centre. Granulation tissue slightly edematous. Numerous mature fibroblasts. A few lymphocytes. Few blood vessels. No foreign body giant cells.	Granulation tissue loose, rich in blood vessels, with collagenic connective tissue fibres in periphery, poor in lymphocytes. Fibrin around suture. No foreign body giant cells (Fig. 4).	Wide granulation area. Centrally, around suture some fibrin and slight edema. Moderate vascularisation. Numerous mature fibroblasts. No foreign body giant cells. Few lymphocytes (Fig. 2 a.).	Fibrin and loose granulation tissue, highly vascularised, around suture. In periphery, mature connective tissue. A few lymphocytes, no giant cells.
her ma tive the con sue. pro ym fo ant	Scar tissue made up of mature connective tissue, rather rich in cells. A few giant cells in periphery. No inflammatory cells.	Fibrin and fairly loose, moderately vascularised granulation tissue with mature fibroblasts still around suture. In periphery, mature connective tissue capsule. A few foreign body giant cells.	A little fibrin remaining. Granulation tissue made up of mature fibroblasts. In periphery, abundant connective tissue, outside which foreign body giant cells (Fig. 2 b).	Slight edema and some fibrin around suture, surrounded by coarse capsule-like connective tissue. No inflammatory or giant cells.
of ive in ve sts	No sign of inflammation. Scar made up of mature connective tissue.	No sign of inflammation. Connective tissue in scar, poor in blood vessels.	Wide connective tissue scar, poor in cells, with no inflammatory or giant cells (Fig. 2 c).	No sign of inflammation. Scar made up of poorly vascularised connective tissue.



a



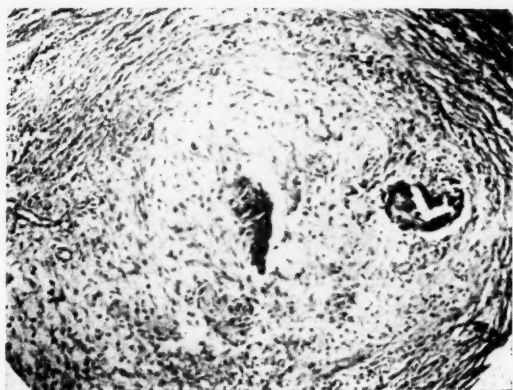
b



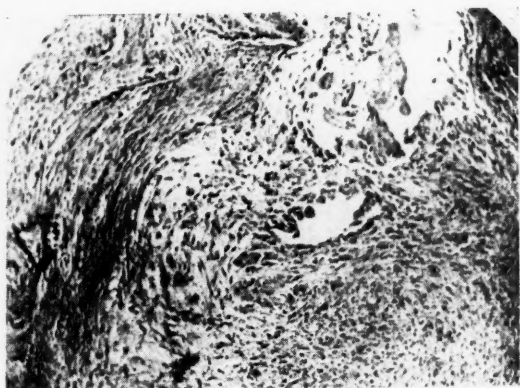
c

Fig. 1. — Catgut suture in the wall of the trachea. a) Ten days after suturation. The suture is surrounded by leukocytes and juvenile fibroblasts, and a few foreign body giant cells. b) Twenty days after suturation. The granulation tissue around the suture is highly vascularised. There are numerous leukocytes. c) Forty days after suturation. An abscess is seen at the suture under tracheal mucosa. Magnification  $\times 72$ .

a



b



c

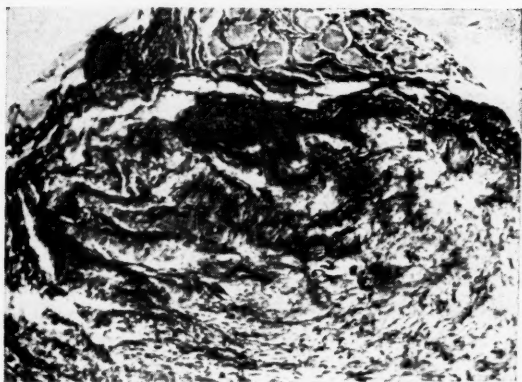


Fig. 2. — Silver-plated steel wire in the wall of the trachea. a) Ten days after suturation. The wire is surrounded by fibrin. The tissue is edematous except in the periphery, in which there are bundles of connective tissue fibres. b) Twenty days after suturation. The wire is surrounded by fibrin, and some edema is present. Round the process, there is a connective tissue capsule. c) Forty days after the suturation. The wire is surrounded by cicatrix made up of connective tissue. Magnification  $\times 72$ .

The table does not reveal any marked differences in the bacteriostatic properties of the suture materials tested. In culture media superior to Fraenkel's medium the differences were even smaller. The results suggested that the bacteriostatic property of silver-plated wire is more distinct than that of the other materials tested. (The results obtained with silk are evidently to be ascribed to the antibacterial substances used for its sterilisation and preservation.)

*Reaction Produced by Suture Materials in Tracheal Tissue.*<sup>1</sup> —

The experiments were carried out on fifteen rabbits, the tracheae of which were sutured with catgut (00; not chromic catgut), silk (No. 3), Mersilene (No. 3/0, Ethicon), nylon (No. 3), steel wire ( $\phi$  0.05 mm), tantalum wire ( $\phi$  0.05 mm), and silver-plated and gold-plated steel wire ( $\phi$  0.05 mm). The sutures were made through all the layers of the trachea. The results were assessed by examining the tissues histologically ten, twenty, and forty days after the suturation. The specimens were fixed in 10 per cent formalin and stained by hematoxylin and eosin or by van Gieson's method. The results are presented in table 2.

The results given in table 2 can be summed up as follows: —

1) Histologically, signs of acute inflammation were present around the catgut sutures all through the experiments, and they were more marked than those found around sutures with other materials. In one preparation, a submucous abscess was detected forty days after suturation. The signs of acute inflammation noted in the vicinity of silk, nylon, and Mersilene sutures were more marked than those noted in the vicinity of metal wires. After forty days, however, no difference was visible in this respect. Neither were any differences detected in the inflammatory processes caused by the various wire sutures. 2) The development of connective tissue took place with equal rapidity around each of the materials tested except that in the vicinity of catgut it was clearly delayed. Silver-plated steel wire produced a distinctly stronger connective tissue reaction than did the other materials; this was quite evident from preparations made forty days after suturation. In the amount of connective

<sup>1</sup> Preliminary experiments were carried out with rabbits and guinea pigs, whose tracheae and main bronchi were sutured. The rabbits proved to be suitable test animals. The part of the trachea which was situated above the jugulum was found to be the most advantageous site for experimental sutures. The tissue referred to in this paper is tissue of this area. The designation «bronchial tissue» also has reference to the tissue of this portion of the trachea.

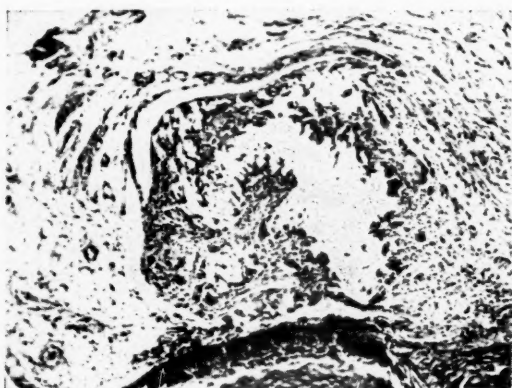


Fig. 3. — Silk suture in the wall of the trachea ten days after suturation. The silk fibres are surrounded by fibroblasts. There are plenty of foreign body giant cells and a few inflammatory cells. Magnification  $\times 72$ .



Fig. 4. — Tantalum wire in the wall of the trachea ten days after suturation. The suture is surrounded by plenty of fibrin. The granulation tissue is edematous. In the periphery of the granuloma, there are bundles of connective tissue fibres. Magnification  $\times 72$ .

tissue produced, gold-plated steel wire took the second place. The material that produced the least amount of connective tissue was Mersilene.

3) Foreign body reaction (by this we mean the presence of foreign body giant cells) was most marked in the vicinity of catgut sutures. The next strongest the reaction was around silk, nylon.



Fig. 5. — Silk suture in the wall of a trachea infected with tubercle bacilli. The photomicrograph was taken six weeks after the suturation. There is some tuberculous granulation tissue and leukocyte infiltration around the suture. The silk fibres are surrounded by fibroblasts and inflammatory cells. Magnification  $\times 72$ .

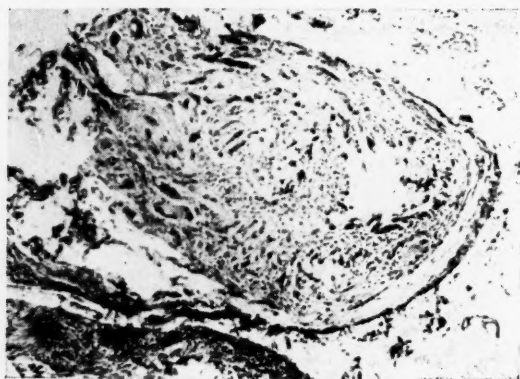


Fig. 6. — Silver-plated steel wire in a tracheal wall infected with tubercle bacilli. The photomicrograph was taken six weeks after the suturation. At the suture, there is a foreign body granuloma separated from the tuberculous granulation tissue by a capsule of connective tissue. Magnification  $\times 72$ .

and Mersilene. The effect of metal wire sutures was fairly uniform and occurred in a mild form only twenty days after the suturation.

*Behaviour of Tuberculous Infection in Bronchial Tissue in the Presence of Different Suture Materials.* — The tracheae of six ether-anesthetised rabbits were sutured in the manner described above (p. 388). After this, some *Mycobacterium tuberculosis* (bovine type)

suspension was spread on the trachea.<sup>1</sup> Six weeks later, all the animals were found to have a strong local tuberculous infection. Histological preparations were made of the tracheae in the same manner as in the previous series.

As in the previous series of experiments, silver-plated steel wire was found to produce the strongest connective tissue reaction. The connective tissue quite clearly separated the wire from the tuberculous granulation tissue. There was also a connective tissue ring around the gold-plated steel wire and the tantalum wire, but it was not quite so distinct as that around the silver-plated wire. The sutures with the other materials tested were in immediate contact with the tuberculous granulation tissue (Fig. 5 and 6).

*Conclusions.* — Tests carried out *in vitro* revealed no marked differences in the bacteriostatic properties of the suture materials. The bacteriostatic effect of silver-plated steel wire, however, seemed to be somewhat more obvious than that of the other materials tested.

The following observations were made on the reactions produced by the suture materials in the tracheal-bronchial tissue: — Catgut produced the most marked signs of acute inflammation; then followed silk, nylon, Mersilene, and plated and unplated steel wire. The foreign body giant cell reaction was strongest around catgut sutures, while around metal wire sutures it occurred later and in a milder form than around sutures with organic materials. The development of connective tissue was simultaneous around all sutures except that around catgut it was delayed. The strongest connective tissue proliferation was caused by silver-plated steel wire, the slightest by Mersilene.

In tracheal tissue infected with tubercle bacilli, tuberculous granulation tissue extended right to the suture made with catgut, silk, nylon, or Mersilene. Metal wire sutures were separated from the tuberculous granulation tissue by a connective tissue layer, which was thickest around silver-plated steel wire sutures.

#### DISCUSSION

The tests carried out *in vitro* revealed no notable differences in the bacteriostatic properties of the suture materials tested. It is true that the tests were made with two bacterial strains only, but

<sup>1</sup> The strain used was obtained from the University Department of Serology and Bacteriology, Helsinki.

Alha (1), among others, has found that different micro-organisms behave much in the same way with respect to one metal. Considering this, we believed it to be unnecessary to carry out experiments with more bacterial strains. It has to be borne in mind, too, that in the present era of antibiotic drugs, tests *in vitro* are of slight significance for the choice of the suture material, even if the bacteriostatic properties of the materials used were much more marked than they really are. The important thing is to find out how living tissue responds to different suture materials and whether tissue involved in a pathological process behaves differently in the presence of different materials. In the work reported here, therefore, emphasis lay chiefly on tests *in vivo*. Histological examination of tissue reactions around the sutures yielded some interesting data. There is reason to believe that the inflammatory reaction, of varying intensity, caused by suturation in the adjacent tissue is an untoward process since it may promote infection of the tissues and delay the development of connective tissue (cf. Localio, Casale, and Hinton, 1943). To assess the significance of giant cell formation is not easy, and supplementary experiments are needed, particularly to show the character of late reactions to wire suturation, for it may be assumed that the elasticity of the suture also plays some part in living tissue. It is also possible that metal wires have later an irritating effect both chemically and galvanically (cf., e.g., Venable and Stuck, 1943). (For this purpose, the maximal observation period used for this study, forty days, is, of course, too short.) It may be an irritation of this kind that is responsible for the connective tissue layer noted around the metal wire, which separated the tuberculous granulation tissue from the suture. It is natural to think that a connective tissue layer of this kind prevents the infection from spreading along the suture. It could be presumed, thus, that, when the danger of a specific infection is imminent, the stimulating effect of metal wire on connective tissue formation adds to the strength of sutures in the larger respiratory passages.

It seems that in living tissue different suture materials have different properties. Unfortunately it does not seem to be possible to decide even from the tissue reaction what would be the best suture material for a given organ in the presence of a given infection. There are, of course, several other criteria available than those studied in the work reported here, such as the tensile strength of

sutured wound (cf., e.g., Localio, Casale, and Hinton, 1943, and Sandblom, 1944). The real value of a suture material can only be found out in prolonged surgical routine work.

#### SUMMARY

The writers carried out experimental studies of the effect of certain suture materials on the respiratory tract.

In tracheal tissue, signs of acute inflammation were most frequently noted in the vicinity of catgut sutures; then followed silk, nylon, Mersilene, and metal wires. Giant cell reaction was also strongest around catgut sutures. In tissue adjoining metal wire sutures giant cell reaction began later than in that adjoining sutures with organic materials. Experiments on tracheal tissue infected with tubercle bacilli showed that tuberculous granulation tissue extended right to the sutures made with organic materials, while a layer of connective tissue around metal wire sutures separated them from the tuberculous granulation tissue. Connective tissue formation was most marked around sutures made with silver-plated steel wire. There is a discussion of the results.

#### REFERENCES

1. ALHA, A. R.: *Acta Pathol. et Microbiol. Scand.* 1946, Suppl. 65.
  2. CAMPBELL, E., MEIROWSKY, A., and TOMPKINS, V.: *Ann. Surg.* 1942:116:763.
  3. KOULUMIES, R.: *Acta Pathol. et Microbiol. Scand.* 1946, Suppl. 64.
  4. LOCALIO, A. A., CASALE, W., and HINTON, J. W.: *Surg., Gynecology and Obstetr.* 1943:77:243, 376 and 481.
  5. SANDBLOM, P.: *Acta Chir. Scand.* 1944; Suppl. 89.
  6. VENABLE, CH. S., and STUCK, W. G.: *Internat. Abstr. of Surg.* 1943:76:297.
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